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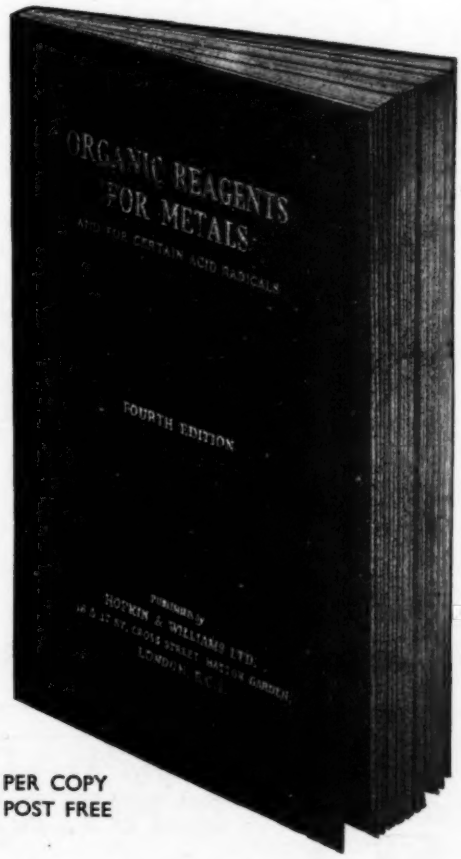

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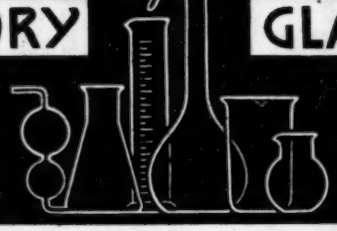

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ERRATA.

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Page.	Line.	
1849	4	for " 2 : 3-*Dimethyl* " read " 2 : 4-*Dimethyl.*"

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534 *The second formula for basseol should be* .

537 *In the figure the numbers* II *and* III *should be interchanged.*

543 20 *The oxidation of methyl siaresinolate by the Oppenhauer reagent* (*see* p. 541) *is as follows :* Methyl siaresinolate (1 g.), aluminium *tert.*-butoxide (3·3 g.), *cyclo*hexanone (10 c.c.), and toluene (50 c.c.) were boiled for 18 hours, the mixture diluted with water and extracted with ether, the extract dried and evaporated, and the residue heated to 100° in a high vacuum. The residue crystallised from dilute acetone in plates, m. p. 214—215°, $[\alpha]_D$ + 57° (*c* = 1·75 in chloroform), giving a yellow colour with tetranitromethane (Found : C, 76·9; H, 10·1. $C_{31}H_{46}O_4$ requires C, 76·8; H, 10·0%).

544 23 *for* " $C_{28}H_{44}$ " *read* " $C_{29}H_{46}$."

545 16* The *dehydro-ester* was prepared as follows : Methyl acetyldihydro-α-elemolate (700 mg.) was boiled for 16 hours with freshly sublimed selenium dioxide (350 mg.) in AnalaR acetic acid (42 c.c.). The solution was evaporated to dryness under reduced pressure, the residue dissolved in ether, and the solution washed with water, sodium hydroxide, again with water, dried, and evaporated. The residue was dissolved in benzene, and the solution percolated through a short column of alumina. The solid recovered on evaporation of the benzene solution crystallised from methyl alcohol in flattened needles (400 mg.), m. p. 128—129° (Found : C, 77·1, 77·4; H, 10·4, 1·02. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%). As the intensity of the light absorption was less than had been expected, the compound was subjected to a lengthy repurification by chromatographic adsorption in petroleum solution, but was recovered unchanged and behaved like a pure compound.

550 41 *for* " β-Elemolic acid " *read* " β-Elemonic acid."

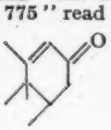
724 23 *for* " 50° " *read* " 5°."

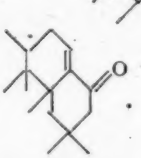
725 9 *for* " *NN'*-dimethyldiacridine " *read* " *NN'*-dimethyldiacridene."

19 *for* " *NN'*-Dimethyldiacridine " *read* " *NN'*-Dimethyldiacridene."

5* for " NN'-*Dimethyldiacridine* " read " NN'-*Dimethyldiacridene.*"

741 22 for " *Helv. Chim. Acta*, 1942, **25**, 775 " read " *Helv. Chim. Acta*, 1942, **25**, 1236."

in formula (III) *ring* D *should be* 

742 *formula* (VII) *should be*  .

750 *add* H *in formula* (I).

752 30* *for* " -*d*-galactoside " *read* " *l*-galactoside."

39* *for* "← " *read* " →" *in bottom row of formulæ.*

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52 34* *for* " below " *read* " above."

80 2 *for* " phosphoric oxide " *read* " phosphoric oxide (14 g.)."

* From bottom.

80. *Rubber, Polyisoprenes, and Allied Compounds. Part IV. The Relative Tendencies towards Substitutive and Additive Reaction during Chlorination.*

By George F. Bloomfield.

It has been previously observed that when chlorine reacts with rubber much hydrogen chloride is evolved, which suggests that the halogenation process may be one of substitution and not of addition at the double bonds. The character of this reaction as it applies to rubber and to other olefins containing the group $\cdot CH_2 \cdot CMe{:}CH \cdot CH_2 \cdot$ has been examined. The main reaction appears undoubtedly to be substitutive, but the products show marked loss of unsaturation as determined by the iodine-value method. This loss is probably partly real, due to the occurrence of some degree of cyclisation, and partly apparent, due to inhibition by the incorporated chlorine atoms of ready iodine chloride addition during the determination.

Although the established mode of reaction of chlorine with unsaturated systems is primarily additive, it has recently been shown by Groll, Hearne, *et al.* (*Ind. Eng. Chem.*, 1939, **31**, 1239, 1413, 1530) that under suitable conditions substitutive reaction may not only occur, but may become the major reaction to the exclusion of addition. According to these authors, high temperatures in the range 300—600° seemed generally to be necessary if substitutive reaction is to predominate, but subsequently it was shown by Rust and Vaughan (*J. Org. Chem.*, 1940, **5**, 472) that a considerable degree of substitutive reaction can be realised at temperatures in the range 130—200° provided certain catalysts which can produce free radicals in contact with the halogen are present.

The polyisoprene system has been found to be particularly susceptible to substitutive attack by oxygen (Farmer and Sundralingam, J., 1942, 116; Farmer and Sutton, *ibid.*, p. 139; Farmer, Bloomfield, Sundralingam, and Sutton, *Trans. Faraday Soc.*, 1942, **38**, 348), and it has long been known that, as judged by the quantity of hydrogen chloride liberated, the chlorination of rubber proceeds largely by substitution in the earlier stages of reaction (McGavack, *Ind. Eng. Chem.*, 1923, **15**, 961). The character of the products thus produced, and the influence of the experimental conditions on the course of reaction, were not, however, studied in any detail by McGavack.

Since the production of hydrogen chloride in the treatment of an olefinic substance with chlorine is no certain indication that true substitution has occurred, new experiments have been made with the object of examining further the character of reaction in the case of rubber and of olefins of allied type. In these experiments attention has been given to the influence of light, oxygen, and temperature on the course of reaction. In order to avoid, as far as possible, complication due to the formation of olefin hydrochlorides through the addition of some of the liberated hydrogen chloride to the olefin, steps have been taken to remove all hydrogen chloride formed in the reaction rapidly from the reaction vessel by means of a stream of inert gas; also the reaction has been conducted at as high a temperature as possible, since the rate of absorption of hydrogen chloride by rubber solutions appears to decrease as the temperature is raised (Bloomfield and Ferry, unpublished observations). The upper limit of temperature has, unfortunately, been much restricted by the necessity of using those solvents which will both dissolve rubber and remain unattacked by chlorine at their boiling points, but even at the comparatively low upper limit thus enforced (77°) it is believed that there is little risk of the undesired hydrochloride-formation occurring.

Conditions of Reaction.—In order to ensure homogeneous chlorination of rubber it has been necessary to work with solutions of the hydrocarbon. The only readily available solvent suitable for use with rubber was carbon tetrachloride which had been freed from disulphide. Chloroform was used in some experiments but proved to undergo substitution too readily to be satisfactory, for, although in the reaction vessel the rubber became chlorinated preferentially to the solvent, yet in the condensing and absorbing systems some chlorination of the solvent vapour carried from the reaction vessel by the effluent gas occurred—especially when the operation was conducted in light—so giving fictitiously high values for the degrees of substitution attained. The chlorinations, except where the influence of oxygen was under examination, were carried out in strict absence of air in the apparatus described on p. 294.

Course of Reaction.—The results obtained under various conditions are summarised in the table, multi-stage chlorinations being regarded as single experiments and so appearing under one number. *If it be assumed that all the hydrogen chloride produced during chlorination arises by substitutive reaction* (and the validity of the assumption is considered later) it can be categorically stated as the result of the experiments that, for all reactions occurring in the dark or in the light, and at temperatures in the neighbourhood of 80°, the dissolved rubber is attacked wholly substitutively by chlorine, $-C_5H_8- + Cl_2 = -C_5H_7Cl- + HCl$, and this applies just so long as the first molecule of chlorine per C_5H_8 unit is being consumed. After this stage additive reaction becomes recognisable (see expts. 10 and 11) and proceeds side by side with substitutive reaction until a second molecule of halogen per C_5H_8 unit has been consumed. When a third molecule of chlorine is brought into reaction (see expt. 10) the process becomes again exclusively substitutive and proceeds with increasing difficulty. Nevertheless, up to the stage represented by the interaction of 2·95 mols. of chlorine per C_5H_8 unit, 84% of the whole of the chlorine is found to have reacted substitutively. With regard to the uniformity or heterogeneity of reaction during the consumption of the second molecule of chlorine, expt. 11 indicates that there is some slight, but not very marked, tendency for the additive reaction to be greater during the consumption of the first half molecule than during that of the second. The reaction product at the stage when slightly more than

Summary of Chlorination Experiments with Rubber.

Expt. No.	Solvent.	Conditions. Light.	Atmos.	Temp.	Chlorine reacted, mols. per C_5H_8.	HCl formed, mols. per C_5H_8.	Substitutive reaction, %.	Product. Cl, %.	I.V.	$[\eta]$.††
1	CCl_4	near-dark	N_2	77°	0·81	0·787	97	30·0	175	—
2	$CHCl_3$	,,	,,	62	0·90	0·884	98	30·4	163	—
								28·2	(insol. portion) 44% of whole	
3	$CHCl_3$	,,	,,	62	1·84	<1·65	<88	49·1	42·5	—
								40·0	(insol. portion) 44·5% of whole	
4	CCl_4	,,	,,	77	1·61	1·16	72	52·8	41·5	—
5	$CHCl_3$	dark	,,	62	1·02	0·988	97	33·9	154	—
								27·1	(insol. portion) 24% of whole	
6	CCl_4	,,	,,	77	1·00	0·973	97	33·6	140	0·416
7	CCl_4	,,	,,	77	1·85	1·29	70	—	—	—
8	CCl_4	light	,,	77	0·83	0·806	96	29·3	173	—
9	CCl_4	dark	,,	77	1·07	0·998	94	—	—	—
		,,	,,	,,	0·54	0·184	34	—	—	—
		light	,,	,,	0·65	0·340	52	—	—	—
10	CCl_4	dark	,,	77	1·07	1·038	96	—	—	—
		light	,,	,,	1·01	0·499	49	—	—	—
		,,	,,	,,	0·58	0·614	100	—	—	—
		,,	,,	,,	0·29	0·318	100	—	—	—
					2·95	2·469	84	64·6	0	—
11	CCl_4	,,	,,	77	1·12	0·973	86	—	—	—
		,,	,,	—	0·46	0·201	44	—	—	—
		,,	,,	—	0·50	0·338	67	—	—	—
					2·08	1·512	72	58·2	0	0·755 ††
12	CCl_4	near-dark	N_2 with trace of O_2	77	1·04	1·012	98	—	—	—
13	CCl_4	,,	much O_2	77	1·0	0·960	96	35·2	117	—
14 *	CCl_4	,,	N_2	77	0·86	0·830	96·5	—	—	insol.
15 †	CCl_4	,,	,,	77	1·04	1·030	99	—	—	insol.
16	CCl_4	,,	,,	−30	1·0	0·822	>82	34·2	129	1·93
17 ‡	none	,,	vac.	−80	0·58	0·230	*ca.* 40	35·9	118	—
18	CS_2	,,	N_2	−80	1·0	0·671	67	34·4	—	—
19	CCl_4 + HOAc	,,	,,	80	0·98	0·921	94	—	—	—
20 §	CCl_4	,,	,,	77	1·18	0·986	84	42·9	—	1·04
21 §	CCl_4	,,	,,	77	1·0	1·00	100	33·8	—	—
22 ‖	CCl_4	,,	,,	77	0·67	0·626	93	27·2	—	—
23 **	CCl_4	,,	,,	77	0·96	0·876	91	37·5	—	1·23
24	CCl_4	,,	,,	77	0·15	0·147	98	6·75	317	—
25	CCl_4	,,	,,	77	0·27	0·259	96	12·3	250	—

* Quinol present. † Phenyl-β-naphthylamine present. ‡ *Dry* crepe rubber employed. § Crepe rubber employed. ‖ Milled crepe rubber employed. ** Gel rubber employed. †† Intrinsic viscosity in benzene + 15% MeOH, except that No. 12 was in benzene alone.

2 mols. of chlorine per C_5H_8 group have reacted at 77° is a tough solid containing 58·2% of chlorine, saturated to iodine chloride, and stable in air. The wholly substituted products obtained by reaction of 1 mol., or less than 1 mol., of chlorine per C_5H_8 group are still somewhat rubber-like, and are stable when kept in evacuated tubes; when, however, they are exposed to air, they evolve hydrogen chloride and become transformed into hard resinous masses with no rubber-like properties. When carbon tetrachloride is replaced by chloroform as solvent much the same course of reaction seems to be pursued, but its details cannot be observed very accurately owing to complications arising from chlorination of the solvent vapours (see above) and from the unavoidable separation of some solid chlorinated material which has a lower chlorine content than the product remaining in solution.

The course of reaction as determined by the quantity of hydrogen chloride evolved, may, therefore, be approximately represented up to the stage at which 3 mols. of chlorine have combined with each C_5H_8 group by the following equations, in which the double isoprene unit is used to avoid introducing half atoms :

$$C_{10}H_{16} + 2Cl_2 = C_{10}H_{14}Cl_2(Cl, 34{\cdot}6\%) + 2HCl$$
$$C_{10}H_{14}Cl_2 + 2Cl_2 = C_{10}H_{13}Cl_5(Cl, 57{\cdot}2\%) + HCl$$
$$C_{10}H_{13}Cl_5 + 2Cl_2 = C_{10}H_{11}Cl_7(Cl, 65{\cdot}4\%) + 2HCl$$

Elementary analyses of the products obtained in expts. 10 and 11 agree closely with the requirements of these equations. Also various published carbon and hydrogen analyses for highly chlorinated rubbers (*e.g.*, cf. Schidrowitz and Redfarn, *J. Soc. Chem. Ind.*, 1935, **54**, 263T) agree as regards the C : H ratio with the formula $C_{10}H_{11}Cl_7$ now advanced, rather than with an earlier formula $C_{10}H_{13}Cl_7$ due to McGavack.

Influence of Light and Temperature.—The exclusively substitutive reaction occurring during the interaction

of the first molecule of chlorine proceeds to all appearance with equal facility in dark or in light (cf. expts. 6 and 8). It is concluded, therefore, that the substitutive type of reaction is in the main uninfluenced by light. The mixed reactions characterising the interaction of the second mol. of chlorine appear to proceed in darkness or in light, but reaction is accelerated by light. In the third stage reaction is slow in the absence of light.

Variations due to change in the temperature of reaction have only been followed over moderate- and low-temperature ranges, since the paucity of suitable solvents has restricted observations (see p. 289). At 80° the course of reaction described above is maintained, and no appreciable change in the dominant substitutive reaction is to be observed as the temperature falls to room temperature. At room temperature and below, however, some difficulty is experienced from secondary reaction, due to the tendency of the liberated hydrogen chloride to combine additively with the rubber; nevertheless, it has been possible to show that at temperatures as low as −30° reaction is still at least 82% substitutive (cf. expt. 16), and at yet lower temperatures, in spite of the fact that the additive reaction of chlorine becomes more pronounced, substitution is still important, amounting to at least 50% at −80°. Chlorination proceeds very readily at these low temperatures and it was even possible to bring to successful reaction a finely divided solid rubber (expt. 18), although here the product was not homogeneous.

The low-temperature chlorination products showed no sign of evolving hydrogen chloride when allowed to warm to room temperature, and indeed certain of the products (cf. expts. 17 and 18) were raised to and maintained at 80° in the absence of air for one hour without any significant amount of hydrogen chloride being evolved. It seems unlikely, therefore, that at the higher temperatures chlorination proceeds by the addition of chlorine at the ethylenic linkages followed by elimination of hydrogen chloride, thus simulating substitutive reaction although in fact it is truly additive.

Influence of Oxygen, Anti-oxidants, and Polarity of Solvent.—Rust and Vaughan (*loc. cit.*) have found that small proportions of oxygen catalyse the substitutive vapour-phase chlorinations of simple olefins at high temperatures, whereas larger proportions inhibit both substitutive and additive reactions—although, strangely enough, the liquid-phase chlorination of *iso*butylene was found to be exclusively substitutive and entirely unaffected by oxygen. In the case of rubber the exclusively substitutive mode of reaction characterising the interaction of the first molecule of chlorine remains unaffected whether a trace or a substantial proportion of oxygen is mixed with the chlorine (cf. expts. 12 and 13), but the products obtained when oxygen was present were of poor stability.

Similarly, the substitutive character of chlorination during the interaction of the first molecule of reagent was unaffected by the addition of the anti-oxidants quinol and phenyl-β-naphthylamine, but the products were again markedly unstable, and appeared to undergo a species of vulcanisation, since, in contrast to the behaviour of the chlorinated rubbers obtained when no anti-oxidants were used, they could not be re-dissolved when once they had been isolated from solution. The vulcanising action of quinol might well be due to the formation of a little tetrachlorobenzoquinone, which has a marked vulcanising action on rubber (cf. Fisher, U.S.P. 1,918,328; Farmer, *Trans. Faraday Soc.*, 1942, **38**, 340), but its cause in the case of the amine is unknown.

Since Kemp (*Ind. Eng. Chem., Anal.*, 1934, **6**, 52) has indicated that the presence of glacial acetic acid is of assistance in suppressing substitutive reaction between iodine chloride and rubber, the influence of this polar solvent has been tested in the chlorination reaction of rubber. No more than a slight inhibitory action on substitution appears to accrue from the introduction of substantial amounts of acetic acid into the solvent used for the chlorination (carbon tetrachloride) since at 80° in near-darkness substitutive reaction to the extent of 94% was observed. The product was again not very stable.

Influence of Chlorination on Molecular Size.—When chlorine is passed into rubber solutions, a marked decrease of viscosity is commonly observed, probably owing to a reduction in the molecular weight of the rubber. Some quantitative observations have been made in collaboration with Dr. Gee, to whom the author's thanks are due. For this purpose the reaction product was isolated from solution, redissolved in benzene plus 15% methyl alcohol, and its intrinsic viscosity $[\eta]$ determined in dilute solution, as described by Gee (*Trans. Faraday Soc.*, 1940, **36**, 1162). It was found that samples of sol rubber having $[\eta] = 2{\cdot}75$ passed when chlorinated at 80° to the point at which each C_5H_8 group had reacted with 1 mol. of chlorine into " mono "-chloro-substitution products $(C_5H_7Cl)_n$, having $[\eta]$ of the order of 0·4. A sample of acetone-extracted crepe rubber of $[\eta] = 2{\cdot}3$ gave a " mono "-chloro-substitution product of $[\eta] = 1{\cdot}04$,* and a sample of gel rubber ($[\eta]$ not measured but probably in excess of 2·5) gave a chlorination product † of $[\eta] = 1{\cdot}23$. In the last two cases degradation was much less severe. The character of the degradation is not yet known with certainty, the phenomenon of molecular-weight reduction appearing not only as the normal and invariable result of autoxidation reaction (cf. preceding papers), but also as a quite usual concomitant of reaction when a variety of reagents are employed with rubber. The apparent degradation effected by chlorine does not appear to extend beyond the stage of " mono "-chloro-substitution, and because in general the phenomenon appears most marked when the sol fraction of rubber is being employed, it seems likely that the phenomenon is an autoxidation effect arising from

* The corresponding value for the molecular weight obtained by osmotic-pressure determination was 240,000, which is to be compared with the value of *ca.* 300,000 to be expected if no degradation had occurred.

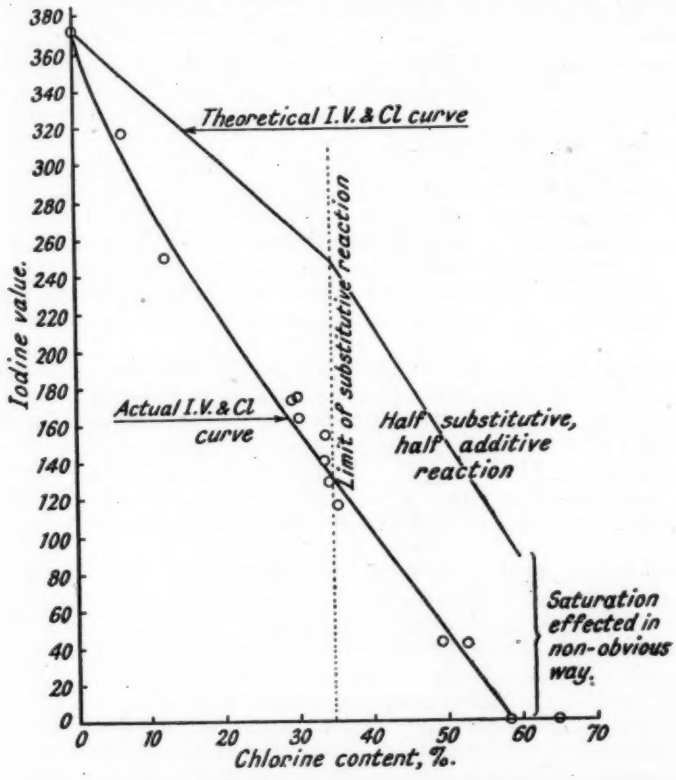
† Probably wholly " mono "-chloro-substituted, so far as the rubber is concerned, but that cannot be fully demonstrated owing to the considerable proportion of non-hydrocarbon impurity present.

small concentrations of oxygen which are either already dissolved or incorporated (as peroxide groups) in the rubber, or gain access in spite of precautions during the preparation and subsequent manipulation of the reaction products. If this explanation is correct, it would be expected that the degradative effect would largely disappear if the chlorination of sol rubber were carried out at low temperatures (*i.e.*, under conditions which decelerate autoxidation) and this is observed in practice, since the " mono "-chlorinated product has then $[\eta] = 1{\cdot}93$. It is of interest that the " mono "-chlorination of gel rubber, like that of sol rubber, seems to be substitutive, although the course of reaction is here obscured owing to the rather high concentration of non-hydrocarbon substances in gel rubber.

The Unsaturation of the Chlorination Products.—In the foregoing account the chief criteria of substitutive reaction have been the production of 1 mol. of hydrogen chloride per mol. of chlorine reacted, the analytical data, and the considerable thermal stability of additive chlorination products where these have been formed. Although there is no evidence to show that under the conditions of reaction (especially the temperature range) adopted there is any considerable production of hydrogen chloride by the *secondary* decomposition of previously

FIG. 1.

The relative tendencies towards substitution and additive reaction during chlorination.



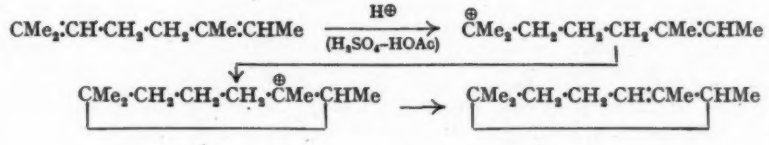
formed, double-bond-addition products, a hypothesis of double-bond re-formation by elimination of hydrogen chloride, either instantaneously as a concomitant of additive reaction of chlorine at a double bond, or from an activated dichloride, cannot be entirely rejected without information as to the precise position of the double bond in the chlorinated molecule. Rust and Vaughan (*J. Org. Chem.*, 1940, **5**, 449) have shown that, although ethyl chloride is stable at temperatures exceeding 415°, yet at 280° in the presence of chlorine it undergoes considerable decomposition to give ethylene and hydrogen chloride. Hence some further check on the reality of the substitutive reaction is desirable. When, however, resort is made to observation of the changes which occur in the unsaturation of the rubber as chlorination proceeds, there is the perplexing discovery that simultaneously with the liberation of hydrogen chloride the unsaturation as measured by the iodine value (I.V.) progressively decreases, and the decrease is sufficiently marked to indicate that (apparently) a very substantial degree of additive reaction has occurred. For instance, for substitutive reaction in which each mono-olefinic C_5H_8 unit passes into C_5H_7Cl, the I.V. should decrease from 373 to 248, but in practice it falls to a value between 130 and 150. It is thus necessary to seek an explanation of the apparent partial saturation of the chlorination products in terms of the invalidity of the iodine method of determining unsaturation as applied to chlorinated rubbers, or else in terms either of the occurrence of cyclisation of the rubber chains due to chlorination, or of the inhibiting influence of the substituent chlorine atom in each C_5H_7Cl group on the reactivity of the double bond. An effect of the last type would fall in line with a vicinal effect of substituent chlorine

reported by Rust and Vaughan (*J. Org. Chem.*, 1941, **6**, 479) whereby the substitution of further chlorine atoms into the system $>CCl\cdot C\leq$ is inhibited by the halogen already present, and with the known low order of additive reactivity of iodine chloride to double bonds containing negative substituents, *e.g.*, dichloroethylene.

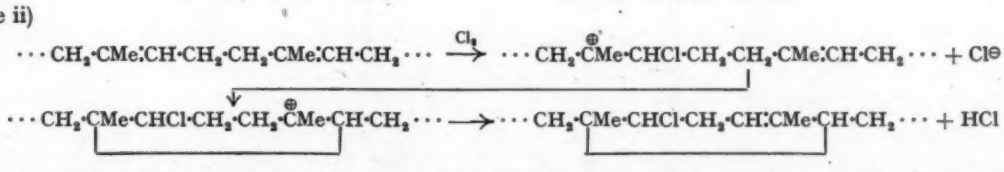
It should be stated that the iodine-value method (Kemp's procedure) has given over a long period results of the most admirable consistency in the determination of the unsaturation of polyisoprenes and other unconjugated polyolefinic systems. Variations in the time of reaction and in the proportion of iodine chloride employed have produced only minor changes in the iodine values observed, so that the above-mentioned anomaly is not to be traced to unsuitable experimental conditions. Peracetic acid, when used as a reagent for determining unsaturation, gave values for chlorinated rubbers substantially lower than those given by iodine chloride. It is, however, significant that when " mono "-chloro-substituted rubbers $(C_5H_7Cl)_n$ reacted additively with further amounts of chlorine, they became saturated (to iodine chloride) at the expected points having regard to their initial unsaturation as determined by the iodine-value method. For instance, " mono "-chloro-rubbers having the theoretical I.V. appropriate to their constitution (248) would be expected to add a whole molecule of chlorine per C_5H_8 group before becoming saturated, whereas " mono "-chloro-products having the commonly-observed I.V. 124 should require to add only half a molecule. The latter requirement is found to be fulfilled in practice, since complete saturation (as measured by the I.V.) of the rubber halide was attained at a total chlorine content of 58·2% (cf. expts. 3, 4, 10, and 11) as the result of a combination of substitutive reaction with $1\frac{1}{2}$ mols. and additive reaction with $\frac{1}{2}$ mol. of chlorine per C_5H_8 unit. During the interaction of the first molecule of chlorine per C_5H_8 group (*i.e.*, during the main substitutive stage) the observed I.V.'s soon began to fall below the theoretical values for homogeneous substitutive reaction; beyond this stage an undoubted additive reaction set in, the decrease in I.V. becoming more rapid. The relationship between chlorine content and I.V. is shown in Fig. 1.

Cyclisation as a Cause of Decreased Unsaturation.—The ease with which the open-chain di-isoprene, dihydromyrcene, cyclises in presence of sulphuric–acetic acid is well known. One double bond in the diolefinic C_{10} unit is lost in this manner, and it is conceivable that reaction between chlorine and rubber could proceed in a comparable manner, *i.e.*, without definite $C_5H_8Cl_2$ units ever being formed. Possible mechanisms for these reactions are set out below:

(Scheme i)



(Scheme ii)



In order to examine this possibility, the behaviour of 1-methyl*cyclo*hexene, dihydromyrcene ($\overline{\overline{5}}$), and squalene ($\overline{\overline{6}}$) towards chlorine has been investigated.

1-Methyl*cyclo*hexene may be regarded as a dialkyl derivative of monomeric rubber, $R\cdot CH_2\cdot CMe:CH\cdot CH_2R$, and it is significant that the product obtained by interaction of the hydrocarbon at 80° with 1 mol. of chlorine retains most of the original olefinic unsaturation and 0·79 mol. of hydrogen chloride is liberated in its formation. The precise degree of unsaturation retained at the end of this stage amounts on an average to $\overline{\overline{5}}_{0\cdot76}$ per mol. of the product as determined by the iodine value; hence the overall reaction appears to be *ca.* 79% substitutive as shown by hydrogen chloride evolution and 76% by iodine-value determination. The product is found, in fact, to consist mainly of chloro-1-methyl*cyclo*hexene admixed with a smaller quantity of 1-methyl*cyclo*hexene dichloride—the latter being a substance of considerable thermal stability. Hence, substitutive chlorination appears to be a major reactivity in the case of this olefin, but it is significant that the unsaturation of the monochloro-substitution product appears to be somewhat lower (I.V., 172) than that calculated (I.V., 180), even when allowance is made for the presence of the dichloride found.

Dihydromyrcene (dimeric rubber) gives in reaction with 1 mol. of chlorine a mixed product, the formation of which is accompanied by the liberation of 0·82 mol. of hydrogen chloride. This was found to consist mainly of unsaturated monochloro-compounds (doubtless several isomeric compounds) from which a fairly pure *monochlorodihydromyrcene* could be isolated. Here, however, the difference between the observed and the calculated values for the unsaturation was more marked (*e.g.*, I.V.: found, 252; calc., 284) than it was with 1-methyl*cyclo*hexene, and a still greater difference was found when 2 mols. of chlorine reacted with dihydromyrcene (*i.e.*, 1 mol. per C_5H_8 unit). In this case the total reaction product, formed with simultaneous liberation of 0·80 mol. of hydrogen chloride, showed an average loss of unsaturation as measured by the I.V. of $\overline{\overline{5}}_{0\cdot28}$, and the pure *dichlorodihydromyrcene* isolated as the major reaction product showed an apparent loss of unsaturation of $\overline{\overline{5}}_{0\cdot22}$ (*e.g.*, I.V.: found, 190; calc., 245). That this effect is due largely, though not entirely,

to a cyclisation mechanism is shown by reduction of the halide, whereby somewhat cyclised dihydromyrcene of I.V. 347 is obtained, whereas if the lowered unsaturation of the dichlorodihydromyrcene had been entirely due to cyclisation, then an I.V. of 285 would have been expected for the regenerated hydrocarbon.

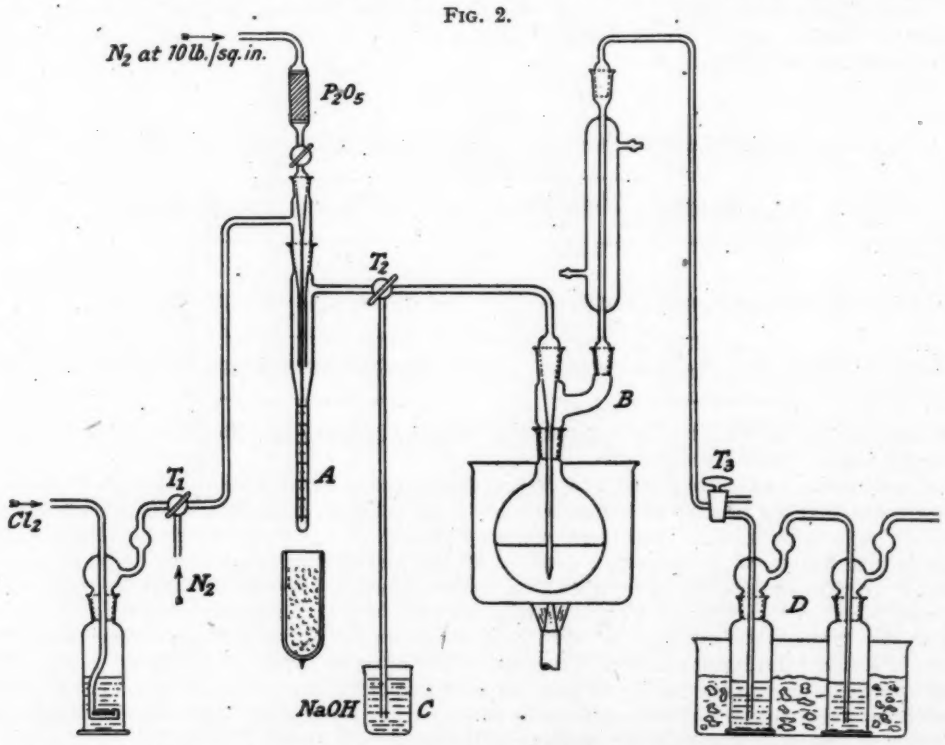
Interaction of squalene with 0·85 mol. of chlorine per C_5H_8 unit liberated hydrogen chloride in quantity corresponding to 90% substitutive reaction, and the unsaturation of the total product as measured by the I.V. fell considerably short of the calculated value (found, 184; calc., 230). Reduction of the halide gave a somewhat cyclised hydrocarbon of I.V. 312 (theoretical value, 372).

The conclusions to be drawn are as follows: (1) Substitutive chlorination of 1-methyl*cyclo*hexene appears to proceed by a direct mechanism, since no question of cyclisation here arises. (2) There is experimental indication that some degree of cyclisation accompanies the chlorination of dihydromyrcene, and if this forms in fact an essential part of the chlorination–cyclisation process represented in scheme ii (and is not merely a subsidiary and independent post-chlorination process due to the catalytic action of hydrogen chloride on the olefinic substance) it appears to be quite insufficient to account for more than a portion of the diminution in unsaturation observed to accompany chlorination. Hence there seems good ground for believing that the chlorination of dihydromyrcene is largely substitutive in character, and that the loss of unsaturation measured by the fall in iodine value is partly a real one to be attributed to cyclisation, but partly an apparent one due to marked steric inhibition of iodine chloride addition at those double bonds which adjoin ·CHCl· or ·CCl_2· groups or which themselves form part of CCl=C groups. (3) In the case of rubber, chlorination is probably in the main truly substitutive, but the cyclisation and the steric factor doubtless both play a part in causing the apparent reduction in unsaturation as measured by iodine value.

Experimental.

Solvents.—Commercial carbon tetrachloride was purified by subjection to exhaustive chlorination in light in presence of a trace of iodine, followed successively by washing with alcoholic sodium hydroxide, then with water, drying over

Fig. 2.



calcium chloride, again chlorinating at the b. p. in light to destroy any trace of alcohol, refluxing over bleaching powder, washing, drying, and distilling over paraffin wax to remove hexachloroethane. The distillate was redistilled over mercury and finally rectified through a good Widmer column. Thus purified, it gave on submission to chlorination at its b. p. in a nitrogen atmosphere only traces of hydrogen chloride. The chloroform and carbon disulphide employed were of "AnalaR" grade.

Rubber.—Except where otherwise stated, the rubber employed was sol rubber,* [η] 2·75, from which the most soluble (and most oxygenated) fractions had been rejected.

Nitrogen.—All nitrogen employed except in two experiments was commercial "purified" nitrogen which had been thoroughly freed from oxygen by passage through Fieser's sodium hyposulphite solution (*J. Amer. Chem. Soc.*, 1924, **46**, 2639).

Apparatus.—This is shown in Fig. 2. The solution of rubber was placed in the flask *B*; then the whole apparatus

* Obtained by diffusion of rubber into light petroleum.

was evacuated *via* stopcock *T*3 and afterwards filled with purified nitrogen *via* stopcock *T*1. A stream of nitrogen (entering through *T*1) was kept circulating through the apparatus throughout the chlorination. The amount of chlorine required for use in any one experiment was condensed in the graduated tube *A*, and this was effected by connecting a chlorine cylinder and sulphuric acid wash-bottle to stopcock *T*1, and applying solid carbon dioxide to *A*; meanwhile the stopcock *T*2 was closed to *B* and opened to the alkali trap *C*. As soon as sufficient chlorine was condensed, the cylinder was disconnected, and all uncondensed chlorine was swept out of the apparatus by a stream of nitrogen. Then *T*2 was re-opened to *B*, and the measured volume of chlorine in *A* caused to volatilise slowly into the rubber solution by allowing the temperature of *A* to rise gradually. The ebullition of the liquid chlorine and the removal from the latter of traces of dissolved oxygen were aided by passing purified nitrogen from a reservoir in which it was confined at 10 lb./sq. in. through a fine capillary jet inserted into *A*. Hydrogen chloride and unreacted chlorine in the effluent gases were absorbed in the system *D* (ice-cooled) containing 2N-sodium hydroxide.

Comparative experiments for determining the influence of light on the course of reaction were conducted in diffused sunlight reinforced by the radiation from a 300-watt incandescent lamp, and in darkness; other experiments were made to test (separately) the influence of oxygen, anti-oxidants, polar liquids, and different temperatures of reaction.

Chlorination of Rubber.—General method. The reaction procedure described above was used. For each determination of the hydrogen chloride and unreacted chlorine present in the absorption system *D*, the solution in the latter (containing chloride, hypochlorite, and excess of sodium hydroxide) was suitably diluted (to 500 c.c.) and an aliquot part (25 c.c.) titrated first with 0·1N-sodium arsenite to determine the hypochlorite present and to convert it into chloride. The total chloride was then determined by acidifying the resulting solution with acetic acid and diluting it to 100 c.c., from which 10 c.c. were taken for titration with 0·025N-silver nitrate, dichlorofluorescein being used as internal indicator (cf. Fajans and Hassel, *Z. Elektrochem.*, 1923, **29**, 495). The trustworthiness of the procedure was tested by a blank run in which no rubber solution was used: 2·66 g. (1·6 c.c.) of liquid chlorine ($d^{-79°}$ 1·663) were vaporised into the chlorination vessel, so giving in the absorption system hypochlorite equivalent to 2·63 g. of introduced chlorine and total chloride equivalent to 2·62 g. of chlorine. No harmful effect due to the traces of carbon tetrachloride which escaped into the absorption system was observed, but in a few experiments in which chloroform was used as solvent or in which somewhat volatile and hydrolysable chlorination products were expected, the absorption system was slightly modified. In these cases two wash-bottles of water were used to absorb the hydrogen chloride, and a third, containing 2N-sodium hydroxide, to absorb the excess of chlorine. The small amount of free chlorine in the first two wash-bottles was determined by the arsenite method, and total chloride by the volumetric method as described above. The hypochlorite in the third wash-bottle was determined separately.

The chlorinated rubber was isolated from solution by precipitation into alcohol, and the adhering solvent removed at room temperature under reduced pressure (finally in a high vacuum). All chlorine contents were determined by the Carius method.

The essential details of the various experiments, not already covered by the table, are set out below. Generally, 5 g. of rubber in 300 c.c. of solvent were used in the reaction flask.

Expt. 1. This gave a soft, pale yellow, rubbery material; yield, 7·1 g. (Found: C, 62·4; H, 7·6; Cl, 30·0; I.V., 175. Calc. for the substitutive reaction of 0·81 mol. of chlorine: C, 62·5; H, 7·55; Cl, 30·0%; I.V., 265). The product rapidly darkened on exposure to air and eventually became hard and dark brown, hydrogen chloride being liberated in the process; when stored in sealed evacuated tubes, however, it remained unchanged.

Expt. 2. The reaction solution (red) contained a considerable amount of insoluble matter, which when separated formed a red solid (3·3 g.) having Cl, 28·2%. The solution yielded a soft, red, rubbery mass (4·2 g.) (Found: Cl, 30·4%; I.V., 163), unstable in air, turning yellow and liberating hydrogen chloride.

Expt. 6. The product of reaction of 1 mol. of chlorine per C_5H_8 group (7·2 g.) had Cl, 33·6; I.V., 140 (Calc. for C_5H_7Cl: Cl, 34·6%; I.V., 248).

Expt. 10. The final product of a chlorination conducted in four stages was a fine, white, stable powder (14·5 g.) having C, 32·1; H, 3·1; Cl, 64·6%; I.V., 0; empirical formula $C_{10}H_{11\cdot3}Cl_{8\cdot3}$.

Expt. 12. The nitrogen used in this experiment contained about 1% of oxygen. The product of reaction of 1 mol. of chlorine per C_5H_8 group resembled that obtained in expt. 6, except that the former was somewhat the less stable.

Expt. 13. Here the nitrogen stream was replaced by a mixture of 60% of oxygen and 40% of nitrogen. The reaction solution was of lower viscosity than usual and yielded a brown product containing no combined oxygen and of such poor stability that hydrogen chloride was liberated even in a vacuum (Found: C, 57·7; H, 7·0; Cl, 35·3%; I.V., 117).

Expt. 16. Sol rubber (5 g.) dissolved in carbon tetrachloride (500 c.c.) was placed in a flask fitted with a stirrer, dropping funnel, nitrogen inlet tube, and an outlet tube connected with the absorption system *D* shown in Fig. 2. The rubber solution was cooled to −30°, and into the cooled solution 181 c.c. of carbon tetrachloride containing 5·3 g. of dissolved chlorine were run as rapidly as possible whilst the hydrogen chloride formed was swept into the absorption system by a brisk current of nitrogen. The reaction mixture (685 c.c.) was poured into sufficient alcohol to give a total volume of 2 l. of which 25 c.c. were titrated with 0·025N-sodium hydroxide. The value of 82% substitutive reaction observed (cf. table) must be regarded as a minimum, since the occurrence of some additive reaction between the hydrogen chloride formed and the unsaturated centres of the rubber cannot be excluded at the reaction temperature employed. The product formed under these conditions by the action of 1 mol. of chlorine per C_5H_8 group was a soft, rubbery mass, similar to that obtained in expt. 6, but more stable (Found: Cl, 34·2%; I.V., 129; [η], 1·93).

Expt. 17. Finely shredded crepe rubber was placed in a tube connected by a stopcock to a 2-l. reservoir, the whole being evacuated to 10^{-4} mm., and the stopcock closed. A tube of chlorine (5 g.) frozen in liquid air, and a flask containing 2N-sodium hydroxide, both fitted with stopcocks, were then attached to the apparatus which was again evacuated and sealed off. The tube containing the rubber was then immersed in solid carbon dioxide, and the chlorine allowed to warm and vaporise therein. Under these conditions it was expected that any hydrogen chloride formed would, on account of its considerable vapour pressure at −80°, volatilise into the 2-l. reservoir from which it could be periodically absorbed in the sodium hydroxide by momentarily opening the communicating stopcock. By operating in this way a total of 2·02 g. of unreacted chlorine and 0·6 g. of chlorine as hydrogen chloride were absorbed, the remaining 3 g. of chlorine having reacted with the rubber, 42% substitutively. The figures thus obtained can only be regarded as approximate. There was no evidence of hydrogen chloride production when the chlorinated product was allowed to warm to room temperature; also, no free hydrogen chloride could be detected after the chlorinated product had been maintained at 80° for an hour. The product was a rather hard, stable substance, but not homogeneous, so the values given for chlorine content and I.V. in the table for this experiment are unlikely to be truly representative of the product as a whole.

Expt. 18. Sol rubber (2 g.), dissolved in carbon disulphide (250 c.c.), was placed in the apparatus used for expt. 16, and cooled to −80°. A solution of chlorine (2·5 g.) in carbon tetrachloride (30 c.c.) was then run in at −70° to −80°. Some insoluble matter at once separated, and some hydrogen chloride was formed. The reaction product was placed in alcohol which had been previously cooled to −80°, the precipitated material was transferred rapidly to more alcohol at −80°, and there manipulated to free it from residual hydrogen chloride, and finally the precipitate was placed in alcohol at room temperature before being freed from solvent at reduced pressure (finally in a high vacuum). The values obtained

(cf. table) pointed to the occurrence of *at least* 67% substitutive reaction. Only mere traces of hydrogen chloride were found in the alcohol in which the product had been placed at room temperature, and in the vacuum system either at room temperature or at 80°, thus indicating that no evolution of hydrogen chloride had occurred on warming the chlorinated rubber from −80° to 80°.

Properties of Monochlororubber, $(C_5H_7Cl)_n$.—The substituted rubbers obtained by the action of 1 mol. of chlorine per C_5H_8 group were generally soluble in benzene and chlorinated hydrocarbons, but the solubility decreased when the specimens were stored, owing to their gradual deterioration. In this deterioration small amounts of hydrogen chloride were liberated over long periods at room temperature, but no high proportion of chlorine was lost ($\gtrsim$0·6% of combined chlorine) when the specimens were heated for 5 hours at 70—80°. The products became more stable after they had been boiled with water, but in this process not more than 3% of combined chlorine was removed, and the products so treated were no longer soluble. Refluxing the products in benzene containing aqueous-alcoholic potassium hydroxide caused a similar diminution in the chlorine content.

On treatment with zinc in boiling benzene containing acetic acid, the products lost all their chlorine, so giving a finely divided, insoluble powder resembling the products commonly formed from rubber by reagents such as stannic, antimony, ferric, and zinc chlorides.

Chlorination of Dihydromyrcene.—Expt. 26. Chlorine (4·6 g.) was passed into dihydromyrcene (3·90 g.; I.V., 364) dissolved in carbon tetrachloride (90 c.c.) at the b. p. of the latter, the apparatus shown in Fig. 2 and the modified absorption system described on p. 295 being used. There were found (*a*) unreacted chlorine 0·73 g., whence reacted chlorine = 3·9 g. (0·98 mol.), and (*b*) chlorine as hydrogen chloride, 1·56 g., corresponding to 80% substitutive reaction. The reaction solution was made up to 100 c.c., and 2-c.c. portions were taken for direct determination of unsaturation of the crude reaction product (Found : I.V., 166. Calc. for 80% substitutive reaction : I.V., 182).

Expt. 27. Chlorine (11·0 g.; 1·01 mols. per C_5H_8 unit) was passed into dihydromyrcene (10·62 g.) in the absence of a solvent at 90—100°. All the chlorine reacted, giving 4·36 g. of hydrogen chloride, corresponding to 80% substitutive reaction. Distillation of the product gave 11 g. of fragrant liquid, b. p. 42—65°/0·05 mm., and 6 g. of a less volatile residue which decomposed below its b. p. The volatile fraction on redistillation at 10^{-3} mm. yielded a small fraction, b. p. 35—46°, and a main fraction, b. p. 46—48°, which proved to be nearly pure dichlorodihydromyrcene (Found : Cl, 33·1; I.V., 202. Calc. for $C_{10}H_{16}Cl_2$: Cl, 34·25%; I.V., 245).

Expt. 28. Chlorine (8·04 g.; 0·5 mol. per C_5H_8 group) was passed into 15·62 g. of dihydromyrcene; 3·3 g. of the chlorine appeared as hydrogen chloride, so corresponding to 82% substitutive reaction. The fragrant liquid product was distilled at 0·2 mm., giving the fractions : (i) b. p. 35—42°, (ii) b. p. 42—44°, (iii) b. p. 44°, (iv) b. p. 44—46°, (v) b. p. 46—48°, (vi) a small residue. The fractions (ii)—(iv) were combined and redistilled at 15 mm., so giving three fractions of b. p. <88°, 88—90°, and 90—92° severally. The middle fraction proved to be fairly pure *monochlorodihydromyrcene* (Found : Cl, 21·4; I.V., 252. $C_{10}H_{17}Cl$ requires Cl, 20·55%; I.V., 294).

Expt. 29.—The formation of dichlorodihydromyrcene (expt. 27) was repeated, freshly prepared dihydromyrcene (I.V., 373) being used. The fractionated product gave *dichlorodihydromyrcene*, b. p. 48—50°/0·01 mm. (Found : C, 58·1; H, 8·1; Cl, 33·85; I.V., 190. $C_{10}H_{16}Cl_2$ requires C, 57·95; H, 7·8; Cl, 34·25%; I.V., 245. The corresponding additive product $C_{10}H_{18}Cl_2$ would require C, 57·4; H, 8·7; Cl, 33·9%; I.V., 123). Reduction of the product with zinc dust and glacial acetic acid at 50—60° gave dihydromyrcene, b. p. 56°/15 mm. (Found : C, 87·0; H, 13·0; I.V., 347. Calc. for $C_{10}H_{18}$: C, 86·9; H, 13·1%; I.V., 373).

Chlorination of Squalene.—Expt. 30. Chlorine (5·1 g.) was passed into a solution of squalene of $n_D^{20\cdot6°}$ 1·4954 (4·68 g.) in carbon tetrachloride (90 c.c.) at the b. p. of the latter, the apparatus shown in Fig. 2 being used. There were found : (*a*) unreacted chlorine, 0·95 g., whence reacted chlorine = 4·15 g. (0·85 mol.), and (*b*) chlorine as hydrogen chloride, 1·86 g., corresponding to 90% substitutive reaction. The chlorinated product was a viscous oil which darkened on exposure to air with liberation of hydrogen chloride (Found : Cl, 33·6; I.V., 184. Calc. for 90% substitutive reaction of 0·85 mol. of chlorine : Cl, 32·8%; I.V., 230). Reduction of the product with zinc dust and glacial acetic acid gave some distillable liquid and an undistillable residue. The former, $n_D^{20\cdot6°}$ 1·4958, was a somewhat modified squalene of lower unsaturation than the original specimen (Found : C, 87·5; H, 12·5; I.V., 313. Calc. for $C_{30}H_{50}$: C, 87·7; H, 12·3%; I.V., 372).

Chlorination of 1-*Methyl*cyclo*hexene.—Expt.* 31. Chlorine (6·25 g.) was passed into a solution of 1-methyl*cyclo*hexene [8·14 g.; I.V., 278 (Calc. : 265)] in carbon tetrachloride (20 c.c.) at the b. p. of the latter, the apparatus shown in Fig. 2 and the modified absorption system again being used. There were found : (*a*) unreacted chlorine, nil, whence reacted chlorine = 1·04 mols.; and (*b*) chlorine as hydrogen chloride 2·74 g., corresponding to 79% substitutive reaction. The unsaturation of the total product was determined directly from an aliquot portion of the reaction solution (Found : I.V., 145. Calc. for 79% substitutive reaction of 1·04 mols. of chlorine : I.V., 150). Distillation of the product gave (i) a little low-boiling material, (ii) a fraction, b. p. 56°/15 mm. (5 g.), and (iii) a fraction, b. p. 50—70°/0·01 mm., but principally 55—60°/0·01 mm. The fraction, b. p. 50—70°/0·01 mm., was saturated to permanganate and to bromine (I.V., 12) and doubtless consisted of the addition product 1-methyl*cyclo*hexene dichloride. Fraction (ii), on the other hand, represented a constant-boiling mixture of this with chloro-1-methyl*cyclo*hexene (7·5% of the former) (Found : C, 63·5; H, 8·45; Cl, 28·3; I.V., 172. Calc. for $C_7H_{11}Cl$: C, 64·35; H, 8·45; Cl, 27·2%; I.V., 193. Calc. for 92·5% of $C_7H_{11}Cl$ + 7·5% of $C_7H_{12}Cl_2$: C, 63·3; H, 8·4; Cl, 28·3%; I.V., 180). Reduction of the constant-boiling mixture (5 g.) with zinc and glacial acetic acid at 60—70° gave 1-methyl*cyclo*hexene, b. p. 109—110°/758 mm. (2·3 g.), contaminated with a trace of chlorinated hydrocarbon (Found : C, 86·65; H, 12·55; I.V., 264. Calc. for C_7H_{12} : C, 87·4; H, 12·6%; I.V., 265), together with a chlorinated compound of b. p. 36—38°/0·01 mm. (Found : C, 69·3; H, 9·5; Cl, 21·1%; I.V., 148).

A further sample of chloro-1-methyl*cyclo*hexene was obtained similarly, but without solvent being used during chlorination. This sample (b. p. 56°/15 mm.) had Cl, 28·3%; I.V., 174.

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81. *On the Theory of Chromatography.*

By Joseph Weiss.

A general theory for the formation of a chromatogram in an absorption column from a single substance and the process of development by a solvent is given and discussed for several adsorption isotherms (Langmuir, Freundlich, linear isotherm).

The structure and shape of the band and the process of elution are discussed in general for the case of complete equilibrium between the solution and the adsorbed state, but some reference is also made to non-equilibrium conditions.

In the following paper an attempt is made to correlate the theory with experimental results.

The method of chromatographic adsorption analysis, discovered by Tswett, is now used mainly for the separation of organic compounds. In its simplest form, a mixture of the compounds in a suitable solvent is allowed to run through a vertical column of a suitable, finely powdered adsorbent. The mixture is separated into a series of bands (chromatogram), which are obvious in the case of coloured substances, and often show fluorescence in U.V. light if they are normally colourless. The separation of these bands can be completed by the process of " development " : after the original chromatogram is formed, one passes a suitable solvent through the column, thereby washing the bands down at different rates and thus effecting their separation. By cutting the column, the bands can be extracted separately, or they can be removed by fractional desorption (elution) with suitable solvents.

It is obvious that for any of these processes the structure of the bands (*e.g.*, width, concentration) is of great interest, for on this must depend primarily the possibility of a separation of two or more substances. Only two published papers deal with the theory of chromatography.* Wilson (*J. Amer. Chem. Soc.*, 1940, **62**, 1583) gives the correct differential equation for the case of complete equilibrium between the solution and the adsorbent, but in the solution of this differential equation and in its physical interpretation he has overlooked various important points, and he therefore obtains the result that the adsorption band—even after development by a solvent—remains always of the same width, depending only on the initial concentration of the solution. Actually, as is well known, the adsorption band broadens out, and also the concentration of the eluate is not in agreement with this theory. Wilson, who was aware of these difficulties, attributed them to secondary effects (finite rate of adsorption and desorption, etc.).

The second paper (Martin and Synge, *Biochem. J.*, 1941, **35**, 1358) only takes into consideration a linear adsorption isotherm, in an adaptation of the theory of the fractionating column.

I. The following theoretical treatment is also based on the assumption of practically instantaneous equilibrium between the solution and the absorbent. The chromatogram of a solution containing a single solute will be discussed first, for this is an essential preliminary to the understanding of the chromatogram of mixtures.

The differential equation governing the adsorption process is given by the conservation condition, *viz.*, that the amount of substance disappearing from a solution of volume dv and concentration c—changing to $(c - dc)$—in passing through an adsorption column of length dx is equal to $-(\partial c/\partial x)dx \,.\, dv$, and this must appear in the quantity adsorbed in the length dx from the volume dv, *i.e.*, $(\partial q/\partial v)dv \,.\, dx$, q being the amount of substance adsorbed per unit of length of the column. Therefore

$$-(\partial c/\partial x)dv \,.\, dx = (\partial q/\partial v)dv \,.\, dx \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

In general,

$$q = Af(c) \text{ (adsorption isotherm)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where A is the amount of adsorbent per unit of length of the column, and

$$\partial q/\partial v = (\partial q/\partial c)(\partial c/\partial v) = Af'(c)(\partial c/\partial v) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

Finally, from (1), (2), and (3) we obtain

$$(\partial c/\partial x) + Af'(c)(\partial c/\partial v) = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

The solution of this equation is given by

$$c = \phi\{v - xAf'(c)\} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

where ϕ is an arbitrary function to be determined by the initial conditions. The full mathematical discussion of equation (5) is given in the Appendix, and we give here only the results there obtained.

II. We consider two operations : (i) When a solution of a given concentration is poured on an adsorption column, and (ii) when pure solvent of volume v is poured on the chromatogram formed according to (i).

(i) According to this theory, the adsorption band formed from a solution of initial concentration c_0 and volume v_0 is of uniform concentration. The amount adsorbed per unit of length (see equation 2) is given by

$$q_0 = Af(c_0) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

* (Note added in proof.) After this paper had gone to press another paper (de Vault, *J. Amer. Chem. Soc.*, 1943, **65**, 532) appeared in which, as far as the same problems are treated, the author arrives at substantially the same conclusions as those reported here.

The width of the adsorption band extending from $x = 0$ to $x = x_0 = l$ is given by

$$v_0c_0/Af(c_0) = l \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

(see Fig. 1) (Appendix, equation A7).

FIG. 1.

Original adsorption band, extending from $x = 0$ *to* $x = x_0 = l$ *(the positive* x*-axis pointing downward).*

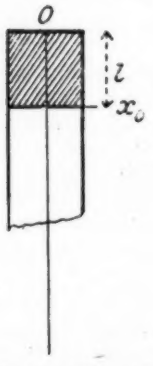
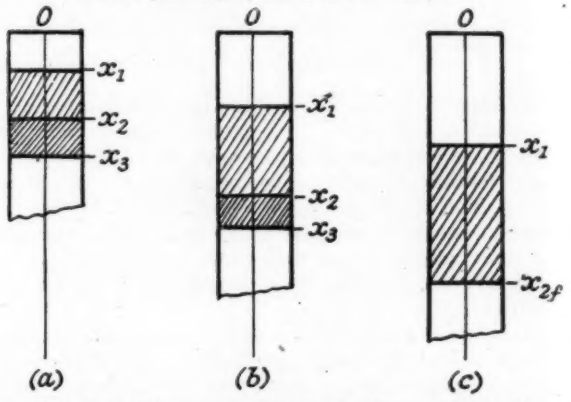


FIG. 2.

Schematical representation of the various stages of development of a single band by a pure solvent.



(ii) The theory shows that in general the band formed originally goes successively through the stages shown schematically by Fig. 2 (*a*), (*b*), (*c*), which follow the stage shown by Fig. 1. When pure solvent is poured on, the band begins to move down and to broaden, "dissolving" from the top end. The band is then, in general, represented by two regions (Fig. 2*a*), *viz.*, the lower region (x_2x_3), where there is still the original concentration present as given by equation (6), and the upper region (x_1x_2) with a variable concentration (c) which is given, in general, as a function of (v/x), *viz.* (see Appendix, equation A16),

$$v/x = Af'(c) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

With increasing v, the region of the original concentration in the lower part gradually diminishes (Fig. 2*b*) and eventually disappears (Fig. 2*c*). The various levels are given by the equations (Appendix, equations A14, A18, A20):

$$x_1 = v/(Af'(0) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

$$x_2 = v/Af'(c_0) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

$$x_3 = vc_0/Af(c_0) + \underbrace{v_0c_0/Af(c_0)}_{l} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

The minimum volume (v_m) of pure solvent to make the lower region of initial concentration just disappear is given by the condition

$$x_2 = x_3 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (12)$$

Substituting from (10) and (11), we obtain

$$v_m = v_0\{c_0f'(c_0)/[f(c_0) - c_0f'(c_0)]\} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (13)$$

III. We shall now discuss the above theory for the known adsorption isotherms.

(a) *Linear adsorption isotherm:*

$$q = Af(c) = ac \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (14)$$

$$Af'(0) = Af'(c_0) = a \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (15)$$

$$x_1 = x_2 = v/a \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (16)$$

$$x_3 = v/a + l \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (17)$$

The width of the band is given by

$$l = v_0/a \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (18)$$

depending only on the initial volume (v_0) and independent of the initial concentration (c_0). In the development, the band moves down unchanged at the constant rate (per unit of volume):

$$x/v = 1/a \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (19)$$

In practice, the case of the linear adsorption isotherm is of little importance. We have discussed this only because, for this special case, our results are identical with those of Wilson (*loc. cit.*).

(b) *Langmuir adsorption isotherm:*

$$q = Af(c) = ac/(1 + bc) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (20)$$

$$Af'(c_0) = a/(1 + bc_0)^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (21)$$

$$Af'(0) = a \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (22)$$

$$x_1 = v/a \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (23)$$

$$x_2 = v(1 + bc_0)^2/a \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (24)$$

$$x_3 = v(1 + bc_0)/a + l \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (25)$$

The equilibrium concentration of the solute in the variable part (x_1x_2) as derived from equation (8) is given by $Af'(c) = v/x = a/(1 + bc)^2$, *i.e.*,

$$c = (\sqrt{ax/v} - 1)/b \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (26)$$

The amount of adsorbed matter (m) in any interval ($x_u x_l$) of the variable part is given by

$$A\int_{x_l}^{x_u} f(c)\mathrm{d}x = m \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (27)$$

Combining this with (26), we have

$$m = a(x_u - x_l)/b - 2\sqrt{av}(\sqrt{x_u} - \sqrt{x_l})/b \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (28)$$

If ($x_u x_l$) represents the whole of the variable interval, *i.e.*, if $x_u = x_2$ and $x_l = x_1$, and if we introduce their values from (23) and (24) into (28), we obtain

$$A\int_{x_1}^{x_2} f(c)\mathrm{d}x = vbc_0^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (29)$$

We can also calculate the total width of the band when all the adsorbed matter present, m_0 (*i.e.*, the total amount), is present in the variable part (*i.e.*, when the lower region has disappeared).

The lower level $x_u = x_{2f}$ can then be obtained from the equations (27) and (28) by introducing the value for x_1 from (23) and we obtain

$$bm_0 = (\sqrt{ax_{2f}} - \sqrt{v})^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (30)$$

and

$$\sqrt{x_{2f}} = \sqrt{v/a} + \sqrt{bm_0/a} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (31)$$

The maximum equilibrium concentration (c_{2f}) at the front edge of the band corresponding to the level x_{2f} is then obtained from equations (26) and (31) as

$$c_{2f}(x_{2f}, v) = \sqrt{m_0/bv} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (32)$$

v being the volume of the pure solvent that has passed.

(c) *Freundlich adsorption isotherm:*

$$q = Af(c) = Bc^{\alpha};\ 0<\alpha<1 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (33)$$

$$Af'(c_0) = \alpha Bc_0^{(\alpha - 1)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (34)$$

$$Af'(0) = \infty \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (35)$$

$$x_1 = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (36)$$

$$x_2 = v/\alpha Bc_0^{(\alpha - 1)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (37)$$

$$x_3 = v/Bc_0^{(\alpha - 1)} + l \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (38)$$

The fact that $x_1 = 0$ means that in this case the upper edge of the band never moves completely away from the top of the column. The equilibrium concentration of the solute in the variable part (x_1x_2) is again given by equation (8) as

$$c = (\alpha Bx/v)^{1/(1 - \alpha)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (39)$$

The amount of adsorbed matter in any interval of the variable part can be calculated from (27) and (39).

The total width of the band, when all the adsorbed matter (m_0) is present in the variable part of the band, can be calculated from the equation

$$A\int_0^{x_2} f(c)\mathrm{d}x = m_0 = \frac{B^{1/(1 - \alpha)}\alpha^{\alpha/(1 - \alpha)}(1 - \alpha)}{v^{\alpha/(1 - \alpha)}} x_2^{1/(1 - \alpha)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (40)$$

and

$$x_{2f} = \frac{(1 - \alpha)^{(1 - \alpha)}}{\alpha^{\alpha}} \frac{v^{\alpha}}{B} m_0^{(1 - \alpha)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (41)$$

The maximum equilibrium concentration (c_{2f}) at the front edge of the band corresponding to the level x_{2f} is then obtained from equations (39) and (41):

$$c_{2f} = (1 - \alpha)\alpha m_0/v \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (42)$$

IV. We will now consider the process of elution of the chromatogram of a single substance. If we add pure solvent to the original band, then for any volume greater than v_m (equation 13) the original band is completely "washed out" (except in the case of the linear isotherm, in which the band remains unchanged). This volume v_m is given for the two cases discussed above by

$$\text{(Langmuir)}\quad v_m \geqslant v_0/bc_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (43)$$

$$\text{(Freundlich)}\quad v_m \geqslant v_0\alpha/(1 - \alpha) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (44)$$

If we continue to add pure solvent, eventually the lower edge of the band reaches the bottom of the adsorption column; until then, no solute will have appeared in the eluate.

We shall call the volume of solvent required to shift the adsorption band to the end of the adsorption column

(of total length x_t) the " threshold " volume v_t, and $V_t = v_t + v_0$. The volume v_t is given by the following expressions, obtained from (17), (31), and (41) for $x = x_t$ and by solving for $v = v_t$:

$$\text{(Linear isotherm)}\quad v_t = a(x_t - l) = ax_t - v_0 \qquad (45)$$

$$\text{(Langmuir)}\quad v_t = (\sqrt{ax_t} - \sqrt{bm_0})^2 \qquad (46)$$

$$\text{(Freundlich)}\quad v_t = \frac{\alpha}{(1-\alpha)^{(1-\alpha)/\alpha}} \frac{(Bx_t)^{(1-\alpha)}}{m_0^{(1-\alpha)/\alpha}} = \frac{ks^{1/\alpha}}{m_0^{(1-\alpha)/\alpha}} \qquad (47)$$

s is the total amount of adsorbent in the column $= Ax_t \propto Bx_t$.

Equations (46) and (47) are only valid for fully developed bands (*i.e.*, $v_t \geqslant v_m$); whereas for partly developed bands one obtains

$$v_t' = [x_t A f(c_0)/c_0] - v_0 \qquad (48)$$

The mean concentration of the substance in the eluate ($\bar{c}_e$) for any volume (v) in excess of v_t is given (corresponding to equation 27) by the expression

$$\bar{c}_e = \frac{m}{v} = \frac{A}{v} \int_{v_t}^{v_t + v} f(c) \,.\, dx \qquad (49)$$

after substituting for $f(c)$ for the different adsorption isotherms [*e.g.*, (20) and (26) or (33) and (39)] and using the relations between v_t and x_t (equations 31 and 41).

We give here only the result for the Freundlich isotherm, which has been verified by experiment and is given by

$$\text{(Freundlich)}\quad \bar{c}_e = (m_0/v)\{1 - [v_t/(v_t + v)]^{\alpha/(1-\alpha)}\} \qquad (50)$$

From this equation the mean concentration in the eluate after passage of, *e.g.*, the volume αv_t is then given by the expression

$$\bar{c}_{e(\alpha v_t)} = (m_0/\alpha v_t)\{1 - [1/(1+\alpha)]^{\alpha/(1-\alpha)}\} \qquad (51)$$

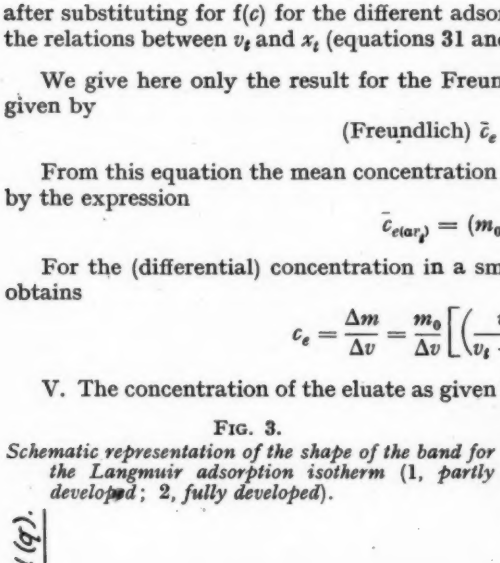
For the (differential) concentration in a small volume Δv, collected after the passage of a volume v, one obtains

$$c_e = \frac{\Delta m}{\Delta v} = \frac{m_0}{\Delta v}\left[\left(\frac{v_t}{v_t + v}\right)^{\alpha/(1-\alpha)} - \left(\frac{v_t}{v_t + v + \Delta v}\right)^{\alpha/(1-\alpha)}\right] \qquad (52)$$

V. The concentration of the eluate as given by (49) or (50) is directly related to that of the adsorbed matter within the band, and the differential concentration of the eluate gives us directly a picture of the " shape " of the band. We shall consider briefly the resultant " shapes " of the bands after development with a solvent for the various adsorption isotherms.

FIG. 3.

Schematic representation of the shape of the band for the Langmuir adsorption isotherm (1, *partly developed*; 2, *fully developed*).



Generally, the amount adsorbed per unit of length of the band is given by $q(x, v)$; and for the variable part (tail) of the band:

$$\partial q/\partial x = A\partial f(c)/\partial x = Af'(c)\partial c/\partial x = (v/x)(\partial c/\partial x) \qquad (53)$$

For the Langmuir isotherm this gives

$$\partial q/\partial x = (2\sqrt{a}/b)(v^{1/2}/x^{3/2}) \quad (\text{for } x \geqslant v/a) \qquad (54)$$

$$q = 0 \quad (\text{for } x \leqslant v/a)$$

This is schematically represented in Fig. 3.

For the Freundlich isotherm we obtain:

$$\frac{\partial q}{\partial x} = \frac{(\alpha B)^{1/(1-\alpha)}}{(1-\alpha)v^{\alpha/(1-\alpha)}} x^{(2\alpha-1)/(1-\alpha)}, \text{ for } \alpha > \tfrac{1}{2} \text{ (Fig. 4}a\text{)} \qquad (55)$$

$$\frac{\partial q}{\partial x} = \frac{(\alpha B)^{1/(1-\alpha)}}{(1-\alpha)v^{\alpha/(1-\alpha)}} \frac{1}{x^{(1-2\alpha)/(1-\alpha)}}, \text{ for } \alpha < \tfrac{1}{2} \text{ (Fig. 4}b\text{)} \qquad (56)$$

The actual amount of substance in the variable (tail) part of the band (m_{tl}) is given by the expressions

$$\text{(Langmuir)}\quad m_{tl} = bc_0^2 v \qquad (57)$$

$$\text{(Freundlich)}\quad m_{tl} = [(1-\alpha)/\alpha^2]c_0 v \qquad (58)$$

and so for small α it is large, and as $\alpha \longrightarrow 1$ it tends to become zero.

In all the above cases we have a sharp frontal edge and the band " dissolves " from the top end. This result is always obtained on the basis of the " equilibrium theory " if $f'(c)$ is a decreasing function of c (see Appendix). Actually, the frontal edge of the band, although often fairly sharp, generally shows some diffuseness, which becomes more marked if there is a strong dependence of the amount adsorbed on the concentration, *e.g.*, in the case where α approaches unity, as for the linear adsorption isotherm. In this case we might expect a deviation from the equilibrium theory at the frontal edge where the concentration is a maximum.

FIG 4.

Shape of the adsorption band in the case of the Freundlich isotherm (1, *partly developed*; 2, *fully developed*).

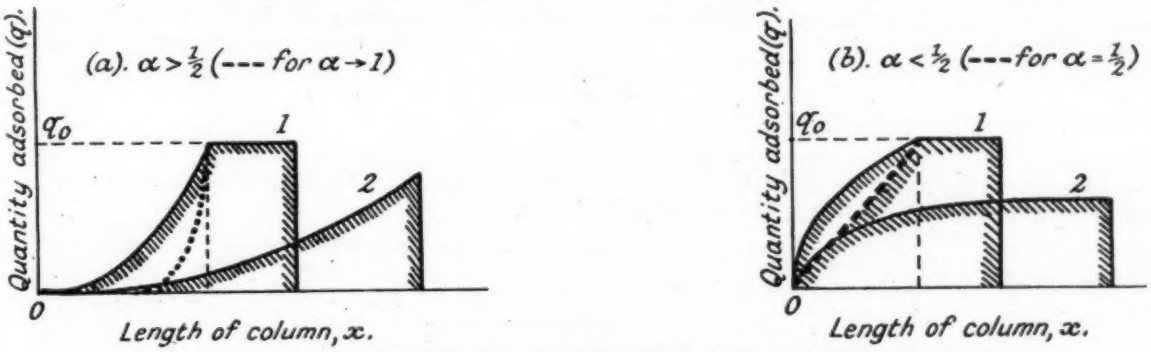


FIG. 4a.—$\alpha > \frac{1}{2}$. *The broken line represents the change in the form if* α *approaches unity.*
FIG. 4b.—$\alpha < \frac{1}{2}$. *The broken line shows the band for* $\alpha = \frac{1}{2}$.

A detailed theory of this effect cannot be given without some assumptions about the kinetics of the processes involved, but the following remarks serve to illustrate this point. In the case of a linear isotherm, the amount adsorbed (per unit of length) is given by $q = ac$ (equation 14). For higher concentrations, if equilibrium is not fully reached, the simple isotherm may be replaced by an equation of the form

$$q = Af(c) = b_1c + b_2(b_1c)^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (59)$$

Here, $b_1 < a$, and the equation must have the property that for sufficiently small c, $b_1 \longrightarrow a$, and the second term disappears. However, at the frontal edge where c is large, we must apply equation (59). This gives

$$q = Af'(c) = b_1 + (2b_2b_1^2)c \quad . \quad . \quad . \quad . \quad (60)$$

In this case $f'(c)$ is an increasing function of c and the concentration in the variable part is given (from the general equation 8) by:

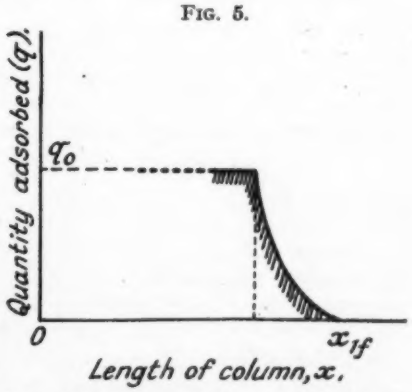
$$c = [(v/x) - b_1]/2b_2b_1^2 \quad . \quad . \quad . \quad . \quad (61)$$

and

$$\partial q/\partial x = -v^2/2b_2b_1^2x^3 \quad . \quad . \quad . \quad . \quad (62)$$

The frontal edge of the adsorption band is schematically represented by Fig. 5.

FIG. 5.



Schematical representation of the " diffuse " frontal edge of an adsorption band for certain non-equilibrium conditions.

The theory was worked out at first to explain certain preliminary results in chromatographic experiments obtained by Weil-Malherbe. There can be no doubt that the assumption of a complete and instantaneous equilibrium and of the absence of all other factors, such as finite rate of adsorption and desorption, hydrodynamic effects, etc., is never completely fulfilled in practice. However, in this treatment only a minimum number of assumptions is necessary and it is of interest to see how far this simple theory does represent the experimental facts. This experimental test is communicated in the following paper.

Appendix.

(1) *Formation of the Chromatogram.*—For equation (5) we have initially

$$\left.\begin{aligned} c(x, 0) &= 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad x > 0 \ (v = 0) \\ c(0, v) &= c_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad v > 0 \ (x = 0) \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (A1)$$

Hence, denoting $v - xAf'(c)$ by ξ, the function $\phi(\xi)$ is defined by

$$\phi(\xi) = \begin{cases} 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad \xi < 0 \\ c_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad \xi > 0 \end{cases} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (A2)$$

Now $\xi < 0$ if $v - xAf'(0) < 0$, *i.e.*,

$$v < xAf'(0) \quad . \quad . \quad .

The third equality gives

$$c = c_0 \text{ for } \beta \leqslant v/x \leqslant Af'(c_0) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (A18)$$

and the fourth equality

$$c = 0 \text{ for } v/x < \beta \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (A19)$$

The lower edge moves down at the same rate as before (as long as there is still some of the original band left), given by

$$v/\beta = vc_0/Af(c_0) + l(= x_3) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (A20)$$

which also defines the value of β introduced in (A18).

I wish to express my sincere thanks to Dr. C. Offord for his help with the mathematical part of the problem, and to Professor G. R. Clemo for his interest in this work.

UNIVERSITY OF DURHAM, KING'S COLLEGE, NEWCASTLE-ON-TYNE, 2. [*Received, April 15th*, 1943.]

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82. *An Experimental Study of Chromatography.*

By H. WEIL-MALHERBE.

Simple chromatographic systems consisting of one adsorbent, one adsorptive, and one solvent were studied by plotting the quantity of adsorptive in the filtrate against the total volume of the filtrate. The resulting "elution curve" is of sigmoid form. The volume of filtrate collected up to the beginning of elution is called the threshold volume, V_t.

It was found that the shape of the elution curve depends on a, the exponential coefficient of Freundlich's adsorption isotherm. Where a is very near to 1, the elution curve is almost symmetrical, whereas a "tail" develops if a is less than 1.

The mean concentration of the eluate was found to be inversely proportional to V_t.

The variations of V_t with factors such as volume and concentration of the original solution or quantity of adsorbent were studied and shown to conform to simple expressions.

The results are discussed on the basis of the theory presented in the preceding paper.

IN spite of the great importance of chromatographic adsorption, it has not yet been systematically studied from a quantitative point of view, and therefore its practical application is still largely empirical. The effect produced by variation of such factors as concentration or quantity of adsorptive, volume of solvent, quantity of adsorbent, etc., upon adsorption and elution is only understood in crude qualitative terms. A quantitative knowledge of these effects is essential for the application of chromatography to quantitative analysis, especially where absence of colour makes direct observation of the band impossible.

The problems which led to the present study arose during the development of a method for the micro-estimation of 3:4-benzpyrene and other hydrocarbons in extracts containing the non-saponifiable matter of animal tissues. In the interest of quantitative recovery the procedure had to be devised in such a way that it was capable of coping with all the concentrations of hydrocarbon likely to occur, down to the limits of accurate measurement; on the other hand, any undue extension of this margin had to be avoided to insure the greatest possible purification. With the aid of the results obtained, it is possible approximately to predict which procedure will best meet these requirements.

One of the reasons for the scarcity of quantitative data on chromatography is the difficulty of measuring the phenomena taking place in the column itself, such as width and concentration of the band and the rate of its advance. All the necessary information can, however, be obtained by measurements carried out on the filtrate. The quantity of solvent necessary to move the band through the whole column is a direct expression of the rate of its progress, and the concentration of the eluate must depend on the concentration of the band in the bottom layer of the column. These two factors thus accurately reflect the structure of the band.

Results.—(1) *The basic experiment: nomenclature.* The simplest chromatographic system has three components: adsorbent, solvent, and substance to be adsorbed. The last will be called the "adsorptive," whether it is in the adsorbed state or in solution. Three phases can be distinguished during the chromatographic process: initial adsorption, final elution, and those continuous processes of elution and readsorption during the advance of the band through the column, which are usually called the phase of development. The solution of the adsorptive *before* adsorption will be called the "initial solution" of initial volume v_0 and initial concentration c_0. The liquid which leaves the lower end of the column will be called the "filtrate," whereas the term "eluate" will refer to only that part of the filtrate that contains adsorptive. The quantity of filtrate which can be collected, before the band has passed the bottom layer of the column and elution proper begins, is termed the "threshold volume," V_t.*

Only simple systems containing a single adsorptive (usually benzpyrene) have been investigated in this study. The solvent used in preparing the initial solution was always the same as that used for development and elution. The adsorbents used were silica gel and aluminium oxide.

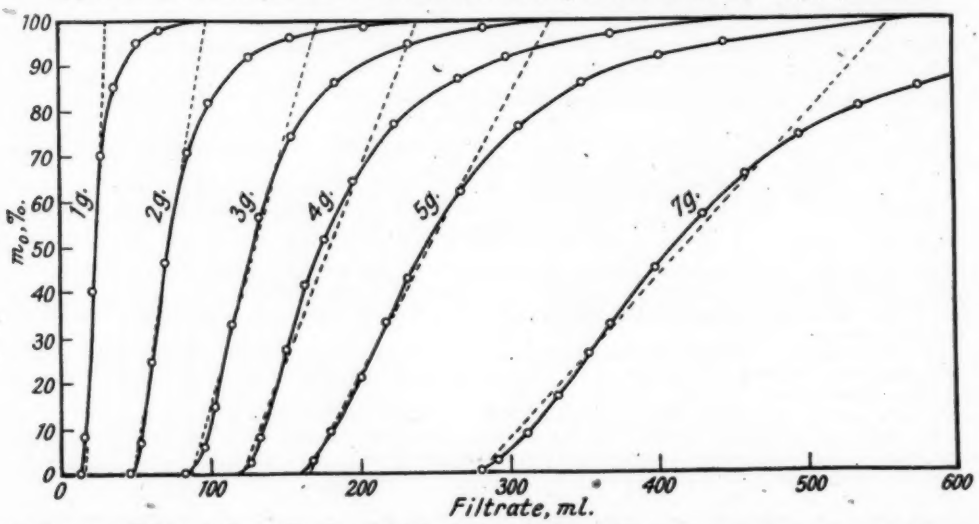
(2) *Elution curves.* If the amount of adsorptive in the filtrate is plotted against the total volume of the filtrate, a curve of typical sigmoid shape results, the elution curve. The concentration of the eluate is given by

* According to this definition, V_t differs from Weiss's term v_t (preceding paper), which equals $V_t - v_0$. As v_0 is usually small compared with V_t, V_t and v_t can be regarded as practically identical in most cases.

the slope of the curve. Roughly, the elution curve may be divided into three parts: a flat part at the beginning and another at the end of the curve, and a steep part in the centre. Actually, there is a gradual transition, the concentration increasing steadily to a maximum and then decreasing again. The rate of elution is never strictly linear, although the curvature is slight in the steep part of the curve. It can therefore be closely approximated by a straight line the slope of which indicates the mean concentration over this particular section of the eluate.

FIG. 1.

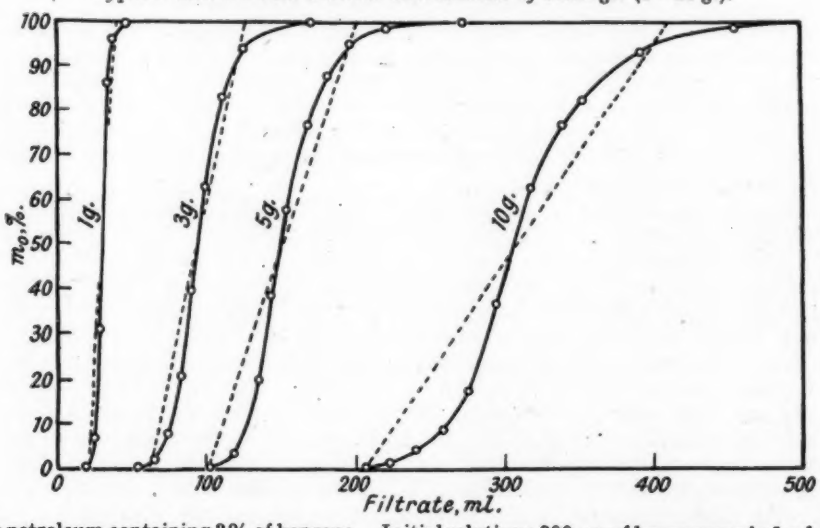
Typical elution curves obtained with columns containing various amounts (1—7 *g.*) *of alumina.*



Solvent: benzene–light petroleum (1 : 4). Initial solution: 300 μg. of benzpyrene in 1 ml. Ratio of length: diameter of columns = 10—15.
The broken lines are m_0/V_t lines.

FIG. 2.

Typical elution curves obtained with columns of silica gel (1—10 *g.*).



Solvent: light petroleum containing 3% of benzene. Initial solution: 300 μg. of benzpyrene in 1 ml. Ratio of length: diameter of columns = 10—18.
The broken lines are m_0/V_t lines.

The more detailed shape of the elution curve depends on the adsorbent used. The curves obtained with alumina (Fig. 1) differ from those obtained with silica gel (Fig. 2) in several respects: in the first case the initial flat part of the curve is so small that it is often hardly measurable. The upper flat part of the curve, on the other hand, is drawn out into a long "tail." The point of inflexion, *i.e.*, the maximum of concentration, is reached after the elution of about 30% of the adsorptive. The steep part of the elution curve may be said to extend over the part showing the elution between 3 and 60% of the adsorptive.

The silica gel curves are more symmetrical. The lower flat part is not much shorter than the upper flat part.

The point of inflexion is slightly below the point marking 50% elution. The section showing the elution of 20—80% of adsorptive represents the steep part of the curve.

(3) *Connection of eluate concentration and threshold volume.* If a number of elution curves with different threshold volumes are compared (Figs. 1 and 2), it appears that the slope, *i.e.*, the mean concentration, of the steep part of the elution curve decreases proportionally to the increasing threshold volume. Let the straight line approximating the steep part of the elution curve be extended up to its points of intersection with the lower and the upper abscissa (the upper one indicating the level of the total quantity of adsorptive, m_0). If the volume of filtrate between the ordinate and the lower point of intersection is called a_1, and that between the ordinate and the upper point of intersection a_2, then the mean concentration over the steep part is given by $m_0/(a_2 - a_1)$. In the case of alumina (Fig. 1) this line is close to a line for which $a_2 - a_1 = a_1 = V_t$. The slope of this line, which will be called the m_0/V_t line, gives the concentration of a solution containing the total amount of adsorptive (m_0) dissolved in the threshold volume (V_t). If this line is superimposed on the elution curve in such a way that it goes through the point of inflexion, it cuts the elution curve at two further points: first at the beginning of the elution curve on the abscissa, *i.e.*, at the point marking V_t, and secondly, at a point marking the elution of about 65% of the adsorptive. It follows that the mean concentration of that part of the eluate containing the first 65% of adsorptive equals m_0/V_t, *i.e.*, that, reckoned from the start of elution, a volume of the eluate $= 0{\cdot}65V_t$ will contain $0{\cdot}65m_0$. The slope of the elution curve indicates that the highest concentration reached during elution is of the order of $m_0/0{\cdot}65V_t$ to $m_0/0{\cdot}8V_t$.

If the curves obtained with silica gel are now considered (Fig. 2), it will be noticed that the divergence between the slope of the steep part of the curve and the m_0/V_t line is greater, the former indicating a maximum concentration of $m_0/0{\cdot}4V_t$ to $m_0/0{\cdot}6V_t$. If now the m_0/V_t line is again superimposed on the elution curve so that it passes through the point of inflexion, it crosses the abscissa at the point marking V_t, as was also the case with the alumina curves. The second point of intersection, however, marks the elution of about 95% of the adsorptive. Up to this point the mean concentration of the eluate $= m_0/V_t$, so $0{\cdot}95V_t$ of the eluate will contain $0{\cdot}95m_0$.

These differences in the shape and slope of the elution curves obtained with alumina on the one hand and with silica gel on the other can be related to the exponential coefficient α of Freundlich's adsorption isotherm, which was found to be 0·67 for the alumina system and 0·96 for the silica gel system. The evidence thus strongly suggests that, in general, the first part, αV_t, of the eluate will contain a fraction, αm_0, of adsorptive.

(4) *Determination of the threshold volume.* The threshold volume has been defined on p. 303. The beginning of elution is marked by the first drop of filtrate containing adsorptive. Owing to the low concentration of the eluate at the beginning of elution, this point is often difficult to ascertain accurately; sometimes it is displaced owing to disturbing influences, such as irregularities of flow. All the values of V_t reported in this study have therefore been determined by a standard method of extrapolation based on the data of a complete elution curve. It has been shown above that an m_0/V_t line joins two points on the lower and upper abscissæ, the latter point twice as far from the ordinate as the former. For any given elution curve that m_0/V_t line is selected which passes through the point of inflexion of the elution curve. Its point of intersection with the abscissa marks the threshold volume. Usually, it coincides with the point where elution has been actually observed to start.

It is obviously much easier accurately to determine by direct observation a point somewhere in the middle of the elution curve. In Table I the values obtained by direct observation for the elution of half the adsorptive are compared with those obtained by extrapolation for the threshold volume. There is a constant ratio between the two sets of values, evidence that the method of extrapolation adopted is not arbitrary but yields correct results; confirmation, too, of the dependence of eluate concentration on V_t.

TABLE I.

Ratio of threshold volume (by extrapolation) to volume necessary for elution of $m_0/2$ *(by observation).*

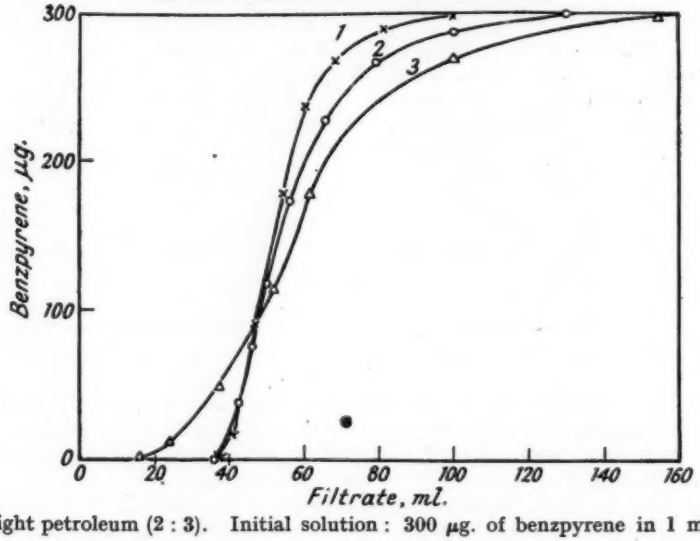
Alumina.						Silica gel.					
V_t (ml.).	$v_{1/2m_0}$ (ml.).	$V_t/v_{1/2m_0}$.	V_t (ml.).	$v_{1/2m_0}$ (ml.).	$V_t/v_{1/2m_0}$.	V_t (ml.).	$v_{1/2m_0}$ (ml.).	$V_t/v_{1/2m_0}$.	V_t (ml.).	$v_{1/2m_0}$ (ml.).	$V_t/v_{1/2m_0}$.
16	23·5	0·681	99	143	0·692	19·5	29·5	0·664	115	172	0·669
49	72	0·680	120	175·5	0·684	40·5	60·5	0·670	117	175·5	0·667
60	89	0·675	122	178	0·685	63	94·5	0·666	145	217	0·669
63	93	0·678	130	191	0·680	101	150	0·674	159	237	0·671
82·5	120	0·688	160	235	0·681	102·5	153	0·670	205	304	0·674
86	126·5	0·680	235	345	0·682	109	162·5	0·671	218	320	0·681
92	133	0·692	280	411	0·682						
97	141	0·688									

(5) *The "typical" elution curve and its limitations.* It follows from Section 3 that knowledge of V_t and α permits the prediction of the approximate shape of the elution curve with the aid of the following three points: (1) origin of the elution curve (abscissa V_t; ordinate 0); (2) upper point of intersection with m_0/V_t line (abscissa $V_t + \alpha V_t$; ordinate αm_0); (3) point of inflexion (abscissa approx. $V_t + \frac{1}{2}\alpha V_t$; ordinate approx. $\frac{1}{2}\alpha m_0$). An elution curve conforming to this general shape may be called a typical elution curve. In order to obtain such a typical curve several conditions must be fulfilled:

(a) Shape of column. This is not a very critical factor. The ratio of length to diameter may be considerably varied (between about 5 and 20) without the elution curve's being affected, provided the quantity of adsorbent be kept constant. If, however, one uses either higher or lower ratios, certain deviations from the shape of the typical elution curve appear (Fig. 3): a very short column leads to flattening of the elution curve, no doubt owing to irregularities of flow caused by decrease of friction towards the centre of the stream, whereas an extremely thin and long column gives some compression of the elution curve, probably owing to the extremely slow rate of filtration, which makes it unsuitable for use.

FIG. 3.

Effect of shape of column on elution curve.

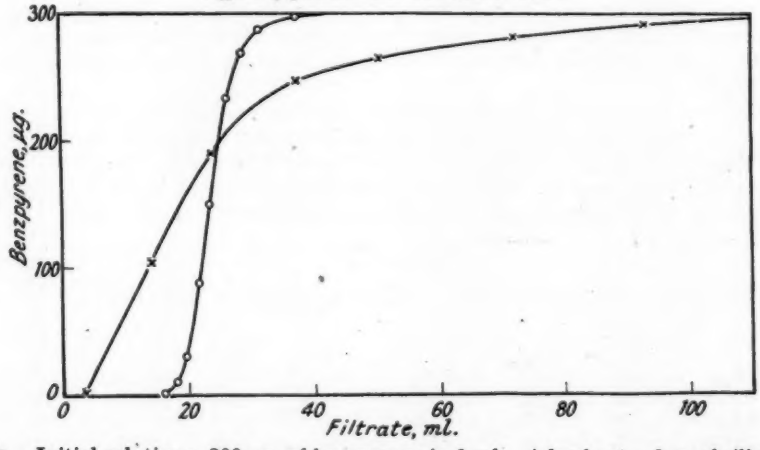


Solvent: benzene–light petroleum (2 : 3). Initial solution: 300 μg. of benzpyrene in 1 ml. Adsorbent: 5 g. of alumina.

Dimensions: column 1 (×——×), 204 × 5 mm.; column 2 (○——○), 70 × 9 mm.; column 3 (Δ——Δ), 20 × 18 mm.

FIG. 4.

Effect of particle size on elution curve.



Solvent: decalin. Initial solution: 300 μg. of benzpyrene in 1 ml. Adsorbent: 3 g. of silica gel.
Column 1 (×——×): 20—30 mesh; 100 × 8 mm. Column 2 (○——○): 100—160 mesh; 80 × 8 mm.

(b) Particle size of adsorbent. The use of an adsorbent of large particle size has an effect on the elution curve similar to that of using a short column, and probably for the same reasons (Fig. 4).

(c) Ratio of v_0 to V_t. The typical elution curve does not contain any part where the rate of elution is linear. The concentration of the eluate may reach a maximum of about $m_0/0{\cdot}4V_t$ with silica gel and $m_0/0{\cdot}65V_t$ with alumina. If this maximum concentration is denoted by m_0/V_t, then the initial concentration c_0 must be $\geq m_0/\delta V_t$. If c_0 is smaller than this, then the elution curve will contain a linear part of concentration c_0 and may be described as incompletely developed. To obtain a fully developed or "typical" elution curve it is therefore necessary that $v_0 \leq \delta V_t$.

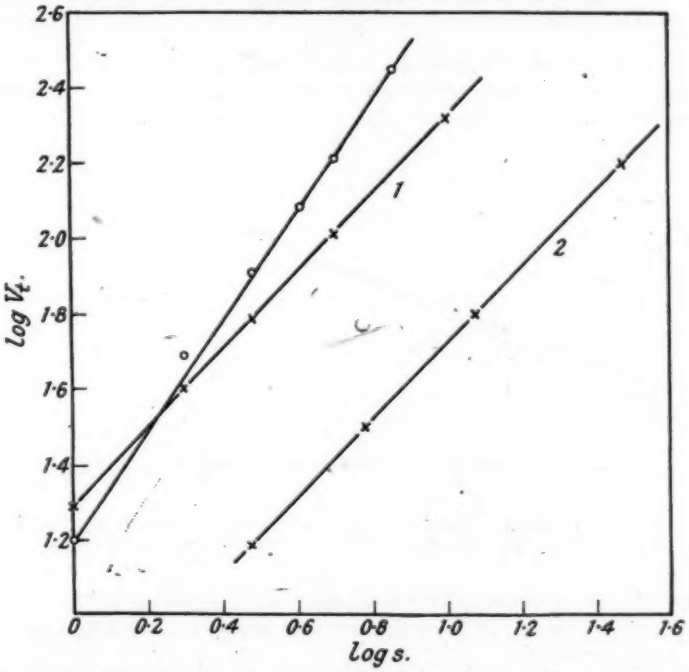
(6) *Variation of* V_t *with the quantity of adsorbent.* As, for any given system of adsorptive, solvent, and

adsorbent, α is constant, the factor which determines the course of elution is V_t. We consider how it is influenced by the variation of some other factors.

If the quantity of adsorbent (s) is varied while other factors are kept constant, again a significant difference between silica gel and alumina is found. If V_t is plotted against s, the relationship is almost linear for the former but parabolic for the latter. On a log scale, straight lines result in both cases (Fig. 5). The silica-gel

FIG. 5.

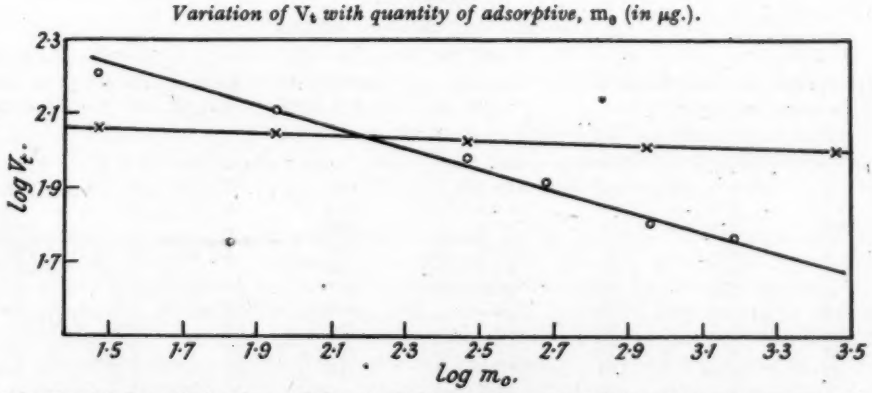
Variation of V_t *(ml.) with quantity of adsorbent,* s *(in g.).*



○——○ : Alumina experiments. Solvent : benzene–light petroleum (1 : 4). Initial solution : 300 μg. of benzpyrene in 1 ml.
×——× : Silica gel experiments. Line 1 : solvent light petroleum containing 3% of benzene. Line 2 : solvent decalin. Initial solution : 300 μg. of benzpyrene in 1 ml.

FIG. 6.

Variation of V_t *with quantity of adsorptive,* m_0 *(in μg.).*



○——○ : Adsorbent 3·5 g. of alumina. Solvent : light petroleum containing 20% of benzene.
×——× : Adsorbent 5 g. of silica gel. Solvent : light petroleum containing 3% of benzene. Initial volume : 15 ml. in each case.

line has a slope of 1·03 and the alumina line a slope of 1·5. It is immediately obvious that these values are the reciprocal of α for the respective systems. We therefore arrive at the equation :

$$V_t = ks^{1/\alpha} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

(7) *Variation of* V_t *with the quantity of adsorptive.* The results of the experiments performed to test this point are presented in Fig. 6, where log V_t is plotted against log m_0. Straight lines are obtained in both sets of

experiments. The line found with alumina has a slope of 0·28, whereas the slope of the silica gel line is 0·03. Denoting this exponent by β, we obtain :

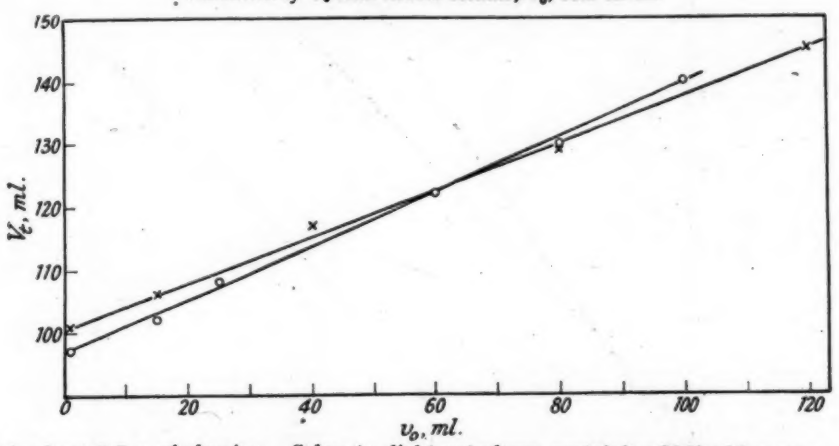
$$V_t = k'/m_0^{\beta} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

(8) *Variation of* V_t *with the initial volume.* The threshold volume was found to increase to some extent with increasing v_0, all other factors including m_0 being kept constant. The relationship is linear, and the slope of the v_0–V_t curve has been determined as 0·43 for alumina and 0·37 for silica gel (Fig. 7). If this factor is given the symbol γ, we may write :

$$V_t = k'' + \gamma v_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

FIG. 7.

Variation of V_t *with initial volume,* v_0, *both in ml.*



○——○ : Adsorbent 3·5 g. of alumina. Solvent : light petroleum containing 20% of benzene.
×——× : Adsorbent 5 g. of silica gel. Solvent : light petroleum containing 3% of benzene. m_0 = 300 μg. of benzpyrene in each case.

In some of these experiments v_0 exceeded the limit set for the achievement of full development. In spite of this, the values of V_t observed lie on the same line as those where development was complete; γ is thus not affected by the degree of development.

(9) *General expression for* V_t. By combining equations (1), (2), and (3) we obtain

$$V_t = Ks^{1/a}/m_0^{\beta} + \gamma v_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

and

$$K = m_0^{\beta}(V_t - \gamma v_0)/s^{1/a} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

TABLE II.

Calculation of threshold volume.

(1) *Alumina.*

Solvent : light petroleum containing 20% of benzene. Adsorptive : benzpyrene, $1/a = 1·5$, $\beta = 0·28$, $\gamma = 0·43$, $K = 70·5$.

			V_t (ml.).					V_t (ml.).	
m_0 (μg.).	v_0 (ml.).	s (g.).	Found.	Calc.	m_0 (μg.).	v (ml.).	s (g.).	Found.	Calc.
30	1	3·5	155	178·5	300	1	2	49	40·8
30	15	3·5	160	184·5	300	1	3	86	74·7
90	15	3·5	126	137·5	300	1	4	120	114·5
120	4	3·5	117	122·7	300	1	5	165	160·0
300	1	3·5	97	94·0	300	1	7	280	264·5
300	5	3·5	99	95·6	480	16	3·5	82·5	89·0
300	30	3·5	108	106·4	900	15	3·5	63	75·2
300	60	3·5	122	119·3	1500	15	3·5	60	66·0
300	80	3·5	130	127·9	1500	60	3·5	87	85·4
300	100	3·5	140	136·5	1920	64	3·5	80·5	83·1
300	1	1	16	14·7					

(2) *Silica gel.*

Solvent : light petroleum containing 3% of benzene. Adsorptive : benzpyrene, $1/a = 1·03$, $\beta = 0·03$, $\gamma = 0·37$, $K = 22·9$.

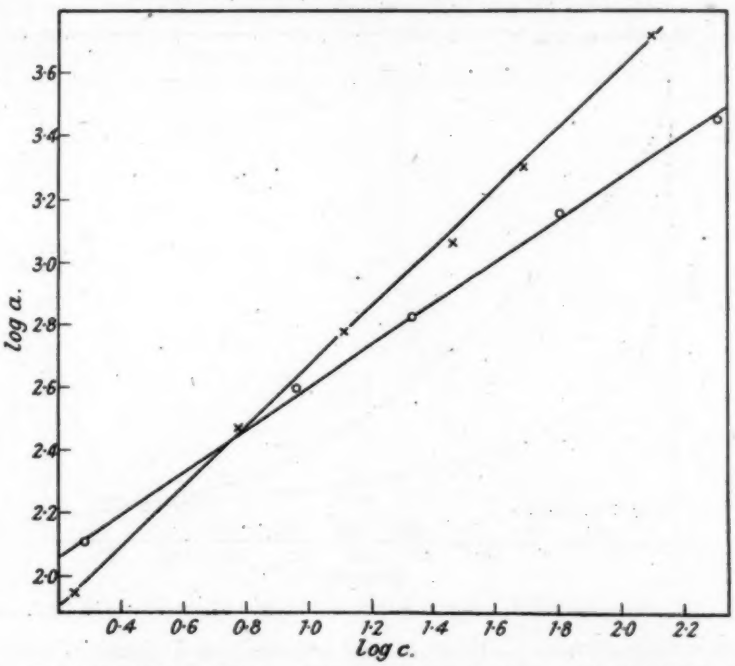
30	15	5	115	114·1	300	15	5	106	106·8
90	15	5	109	110·5	300	40	5	117	116·1
300	1	1	19·5	19·7	300	80	5	129	130·9
300	1	2	40·5	39·9	300	120	5	145	145·6
300	1	3	63	60·3	900	15	5	103	103·9
300	1	5	101	101·7	3000	15	5	102	100·0
300	1	10	205	207·9					

In a system for which the constants α, β, and γ are known, it is sufficient to determine K in a single experiment; from this the approximate value of V_t can be calculated for any possible values of m_0, V_0, and s. Table II shows the degree of agreement between calculation and observation. This is more satisfactory with silica gel than with alumina, which may partly be due to the fact that the alumina experiments were done with long time intervals, so that different batches of solvents had to be used and, although the adsorbent was from the same batch, it had to be dried and stored in different portions.

(10) *Freundlich's adsorption isotherm.* The results already reported are based on the experimental data contained in Fig. 8. Here, a = amount adsorbed per unit weight of adsorbent (μg./g.) and c = the final concentration of the supernatant solution (μg./ml.). The correlation between a and c is given by $a = k'''c^a$.

FIG. 8.

Adsorption isotherms (concns. in μg. of benzpyrene/ml.).



○——○ : Adsorbent, alumina. Solvent: light petroleum containing 20% of benzene.
×——× : Adsorbent, silica gel. Solvent: light petroleum containing 3% of benzene.

(11) *Results with other adsorptives.* The general validity of the results obtained with benzpyrene was checked in a series of experiments in which two other adsorptives, *p*-dimethylaminoazobenzene and acridine, were used. Alumina was the adsorbent, and benzene the solvent. The correlation between V_t and the shape and slope of the elution curve was the same as in the experiments with benzpyrene and alumina. Also the variation of V_t with the quantity of adsorbent was investigated, and the exponential coefficients found (1·45 and 1·48) were about the same as with benzpyrene (1·5). Details of these experiments are omitted for the sake of economy.

Some Practical Applications.—(1) *Determination of eluant power.* If in a chromatographic system the only factor to be varied is the solvent, the value of V_t found may serve as a measure of the eluant power for which so far no quantitative data are available. The relative eluant powers of light petroleum, benzene, and mixtures of both are recorded in Fig. 9, where $k = V_t/s^{1/a}$. The ×——× line shows the values to be expected from a purely additive behaviour of the mixtures; they considerably diverge from the observed values, showing that the addition of light petroleum decreases the eluant power of benzene far beyond what can be attributed to mere dilution.

(2) *Standardisation of adsorbents.* Two different batches of the same adsorbent may differ appreciably with respect to their adsorbing qualities. A problem which frequently arises on changing over from one batch of adsorbent to another is to find out how much of the new adsorbent will make up a column of adsorbing power equal to a given column of the old adsorbent. Provided m_0 and v_0 be kept constant, formula (1) is valid, from which it follows that $k = V_t/s^{1/a}$. A series of adsorbents can be standardised by determining their k values, *i.e.*, the threshold volumes per unit weight of adsorbent. For two different adsorbents having k values of k_1 and k_2 we obtain $k_1/k_2 = (s_2/s_1)^{1/a}$ and $s_2 = s_1(k_1/k_2)^a$ where s_1 and s_2 are the respective quantities of adsorbent.

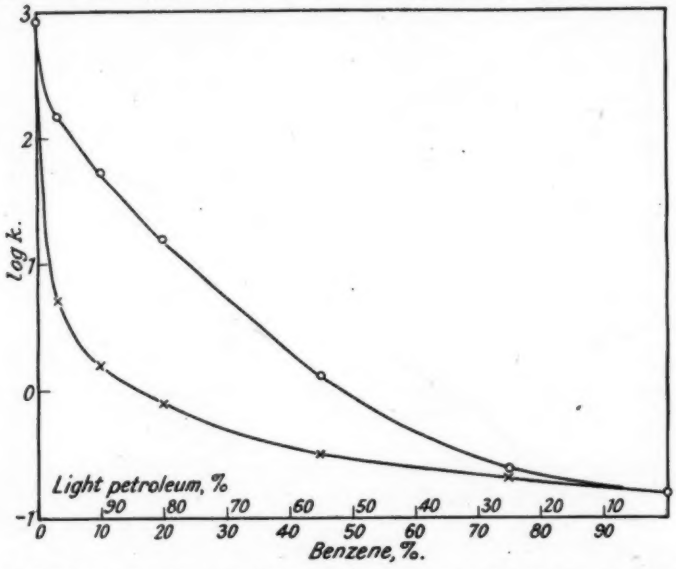
(3) *To determine the minimum size of a column necessary to ensure full development of an initial solution of* v_0, m_0. As previously found [Section 5(c)], the condition for full development is that $v_0 < 0{\cdot}65V_t$ in the case

of alumina, and $v_0 < 0{\cdot}4V_t$ in that of silica gel. Allowing a margin of safety, we may put $V_t = 3v_0$ for silica gel and $V_t = 2v_0$ for alumina as the limiting condition for full development. From equation (5) it follows that, for silica gel, $s = [m_0^{\beta}v_0(3-\gamma)/K]^{\alpha}$, and for alumina we have the same expression but with $(2-\gamma)$ instead of $(3-\gamma)$.

(4) *To determine the fraction of the filtrate containing the eluate.* We now give the solution of some problems connected with the micro-estimation of benzpyrene. The method adopted consisted of the following steps: adsorption on a column of silica gel from light petroleum, and elution with light petroleum containing 20% of benzene; re-adsorption on a column of alumina from this mixture, followed by development with the same solvent; elution with benzene. The method had to be so devised that it allowed quantitative recovery of 0·5—300 μg. of benzpyrene. Both columns contained 2 g. of adsorbent.

FIG. 9.

Variation of k (*threshold volume per g. of adsorbent*) *with composition of light petroleum–benzene mixtures.*



Adsorbent, alumina. Initial solution, 300 μg. of benzpyrene in 1 ml.

The first adsorption took place from 5—10 ml. of light petroleum on a column of silica gel; v_0 is here only 2·5—5% of V_t and can therefore be neglected in the calculation of the next step, *viz.*, elution by benzene–light petroleum (1 : 4). From formula (4) we obtain for $m_0 = 300$ μg. and $K = 2{\cdot}41$

$$V_{t(300\ \mu g.)} = 2{\cdot}41 \times 2^{1{\cdot}03}/300^{0{\cdot}03} = 4{\cdot}15 \text{ ml.}$$

and for $m_0 = 0{\cdot}5$ μg.:

$$V_{t(0{\cdot}5\ \mu g.)} = 2{\cdot}41 \times 2^{1{\cdot}03}/0{\cdot}5^{0{\cdot}03} = 5{\cdot}02 \text{ ml.}$$

With silica gel, elution is completed after the total filtrate has reached a volume of about $2{\cdot}5V_t$. The portion of the filtrate containing the eluate is therefore between 4·15 and 12·6 ml. A margin of safety being allowed, the first 15 ml. of the filtrate were collected, and passed through the alumina column. For $K = 78{\cdot}8$ and $m_0 = 300$ μg. we find:

$$V_{t(300\ \mu g.)} = 78{\cdot}8 \times 2^{1{\cdot}5}/300^{0{\cdot}28} + 0{\cdot}43 \times 15 = 51{\cdot}8 \text{ ml.}$$

and for $m_0 = 0{\cdot}5$ μg.:

$$V_{t(0{\cdot}5\ \mu g.)} = 78{\cdot}8 \times 2^{1{\cdot}5}/0{\cdot}5^{0{\cdot}28} + 0{\cdot}43 \times 15 = 277 \text{ ml.}$$

With alumina a volume of filtrate of at least $4V_t$ has to be collected before elution is completed; the required fraction is therefore that between 50 and 1200 ml. of filtrate. After the collection of the first 50 ml. of filtrate, which were rejected, elution was carried out with pure benzene. For $K = 0{\cdot}79$, the required fraction is that between 0·4 and 12 ml.

Such calculations are, of course, only an approximation, subject to the absence of any appreciable displacement effects due to impurities. They have to be verified by direct observation.

DISCUSSION.

The results obtained show that a number of features of chromatography conform to simple empirical expressions. The question arises how these compare with the theory developed on the assumption of immediate and complete adsorption equilibrium (Weiss, preceding paper).

The variation of the threshold volume V_t with the amount of adsorbent s is given by $V_t = ks^{1/\alpha}$, where α is

the exponential coefficient of Freundlich's adsorption isotherm. In this case the experimental results were so unequivocal ($1/\alpha$ for alumina: calc. 1·5; found, 1·5; for silica gel: calc., 1·04; found, 1·03) that the correlation with the adsorption isotherm immediately suggested itself. It also follows directly from the theory (Weiss, equation 47). For the case of the linear adsorption isotherm V_t is directly proportional to the quantity of adsorbent.

On the other hand, the factor γ, which determines the variation of V_t with v_0, was found to be almost identical for both adsorbents despite the difference of their adsorption isotherms, and it might therefore be assumed that γ is independent of α. This, too, is confirmed by the theory (Weiss equations 47 and 48) which postulates $\gamma = 1$ for complete development ($v_0 < \delta V_t$) and $\gamma < 1 > 0$ for incomplete development ($v_0 > \delta V_t$). Actually, γ was found to be about 0·4 regardless of the degree of development. If v_0 is very small compared with V_t, then the effect of v_0 on V_t becomes insignificant.

FIG. 10a.

Effect of the adsorption isotherm on the elution curve on the basis of the experimental findings.

FIG. 10b.

Effect of the adsorption isotherm on the elution curve on the basis of the theory.

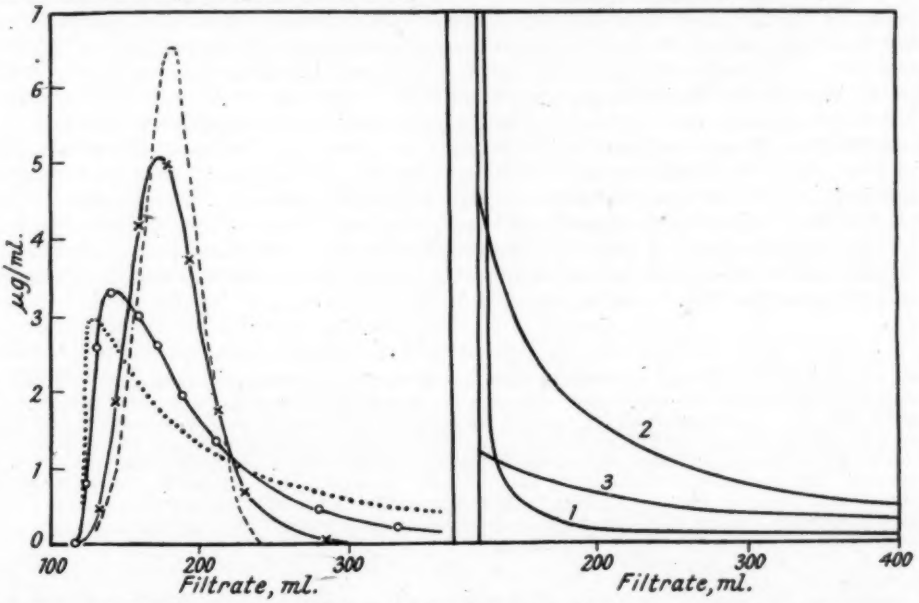


FIG. 10a.—○——○: Elution curve with 4 g. of alumina; $v_0 = 1$ ml., $m_0 = 300$ μg. of benzpyrene. Solvent: benzene–light petroleum (1 : 4).
×——×: Elution curve with 6 g. of silica gel; $v_0 = 1$ ml., $m_0 = 300$ μg. of benzpyrene. Solvent: light petroleum containing 3% of benzene.
Broken line: Elution curve extrapolated for $\alpha = 1$.
Dotted line: Elution curve extrapolated for $\alpha = \frac{1}{2}$.

FIG. 10b.—Curve 1: Elution curve for α near 1.
Curve 2: Elution curve for $\alpha = \frac{2}{3}$.
Curve 3: Elution curve for $\alpha = \frac{1}{2}$.

The exponential coefficient, β, giving the dependence of V_t on the quantity of adsorptive (m_0), is different in the two cases, and it is therefore probable that it is a function of α. According to the theory (Weiss, equation 47), $\beta = (1 - \alpha)/\alpha$. The agreement is good in the case of silica gel, where $\beta = 0{\cdot}03$ and $(1 - \alpha)/\alpha$ is 0·04, but less satisfactory for alumina, where $\beta = 0{\cdot}28$ and $(1 - \alpha)/\alpha = 0{\cdot}50$. For a system with a linear adsorption isotherm, $(1 - \alpha)/\alpha$ would be 0; here V_t is independent of m_0 or $c_{0,\ \text{const. } v_0}$. This is practically realised with silica gel, where V_t was found to be 115 and 103 ml. with initial concentrations of 30 μg. and 3000 μg./ml., respectively, *i.e.*, a decrease of 10·5% for a hundredfold increase of concentration.

The shape of the elution curve to be expected on the basis of the equilibrium theory can be predicted with the aid of equations (50 and 52, Weiss), which only apply to those cases where development has been complete For the linear adsorption isotherm, $c_{el.} = c_0$; this means that in this case no development takes place: the concentration of the eluate is that of the original solution, m_0/v_0.

If α is near 1, the factor $[v_t/(v_t + v_{el.})]^{\alpha/(1-\alpha)}$ (Weiss, equation 50) is negligibly small. In such a case, the effect of development will be small, and the concentration of the eluate should not differ greatly from that of the original solution; also, the "tail" at the end of elution will be small. Such a case is illustrated by curve 1, Fig. 10b. Curve 2 shows a theoretical elution curve for $\alpha = \frac{2}{3}$, and curve 3 one for $\alpha = \frac{1}{2}$. It will be noticed that the "tail" of the elution curve increases, and the change of concentration during elution becomes less marked with decreasing α. The maximum of concentration is always reached at the very beginning of elution.

On the experimental side, two points were found up to which the mean concentration of the eluate was m_0/V_t, *viz.*, those marking volumes of eluate of $\frac{1}{2}\alpha V_t$ and αV. In spite of the considerable difference of their adsorption isotherms, the elution curves of both silica gel and alumina conformed to this rule. It is therefore perhaps permissible to assume that this relation holds for all values of α. For $\alpha = 1$, it follows on this assumption that the mean concentration of the entire eluate is m_0/V_t, or that the quantity of solvent required for complete elution equals $2V_t$, if reckoned from the beginning of the adsorption phase, or V_t, if reckoned from the beginning of the elution phase.

The maximum of concentration was found to lie near the point corresponding to a volume of eluate $= \frac{1}{2}\alpha V_t$, for both the silica gel and the alumina curve. For the case of $\alpha = 1$, it might be expected to coincide with the point where $\frac{1}{2}m_0$ are eluted. Here the elution curve would be strictly symmetrical.

Fig. 10a shows elution curves obtained by experiments with alumina and silica gel, and also curves for $\alpha = 1$ and $\alpha = \frac{1}{3}$ which were extrapolated on the basis of the assumptions just outlined. To facilitate comparison, the curves of Fig. 10b were calculated by assuming the same experimental conditions as those under which the observations recorded in Fig. 10a were made. Agreement between theory and observation is qualitatively satisfactory for the case of alumina where $\alpha = \frac{2}{3}$; here the mean concentration of the eluate for $\frac{1}{2}\alpha V_t$ is calculated as $1{\cdot}3m_0/V_t$ and for αV_t as $0{\cdot}96m_0/V_t$. The theory is, however, no longer fulfilled in the case of silica gel, where α is near 1. According to the theory the concentration of the eluate is independent of V_t and is equal to c_0, if $\alpha = 1$. The experimental results show for a case where α is very near 1 that the concentration of the eluate is still inversely proportional to V_t. As shown by Weiss (p. 302), a better approximation to the experimental findings is obtained by introducing certain non-equilibrium factors.

The volume of v_0 which must not be exceeded if full development is to be attained has been defined by the formula $v_0 = \delta V_t$. According to the theory (Weiss, equation 44), δ, like β, is equal to $(1 - \alpha)/\alpha$; δ was found to be approximately 0·65 in the case of alumina and 0·4 in that of silica gel. The values calculated from the theory are 0·5 and 0·04 respectively; there is thus reasonable agreement in the first example, but again the theory is no longer obeyed when α is near 1. The theory postulates that in the case of the linear adsorption isotherm $\delta = 0$, *i.e.*, that the volume necessary to bring about development is infinite. The experimental results, however, suggest that here, too, this volume is finite.

Experimental.

Adsorbents.—Silica gel (B.D.H.) was ground, the 100—160 mesh fraction screened out, and dried at 110° for 24 hours without any further purification. It was stored in a glass-stoppered bottle kept in a desiccator over phosphoric oxide. Aluminium oxide (B.D.H., " for chromatographic adsorption analysis ") was dried at 300° for 3 hours and stored as above. During operations, such as weighing or filling the columns, exposure to atmospheric moisture was reduced to a minimum.

Solvents.—High purification of solvents proved indispensable for obtaining reproducible results.

Light petroleum. The commercial liquid (b. p. 40—60°) was purified by the following successive extractions, each carried out with several minutes' vigorous shaking: nitrating mixture (4 times), fuming sulphuric acid (3 times), water, and 25% sodium hydroxide. After being dried over calcium chloride, it was rectified through an efficient column of the Clarke and Rahrs type (*J. Ind. Eng. Chem.*, 1923, **15**, 349).

Benzene. Extraction of A.R. benzene with concentrated sulphuric acid (3—4 times), followed by washing with water and alkali, drying, and rectification.

Decalin. Purified as for benzene. Decalin solutions of benzpyrene must be re-extracted with sulphuric acid before recovery, as benzpyrene is slightly volatile with decalin.

Estimation of Benzpyrene.—3:4-Benzpyrene was estimated fluorimetrically. A G.E.C. Osira lamp served as U.V. light source. Fluorescence was measured by a Westinghouse " Green Sensitive " photoelectric cell set at right angles to the incident beam and connected to a mirror galvanometer. It was mounted on the blackened inside of a closed box. The U.V. beam entered the box through a window of Wood's glass, in front of which was a flat-bottomed spherical quartz flask of about 250 ml. capacity, filled with distilled water, which served as condenser. The solution to be examined was placed in a thin-walled test-tube of soft glass, which was passed through a closely fitting hole in the centre of the lid of the box and held in position by a shallow receptacle at a level with the lower end of the photoelectric cell. Before entering the latter, the fluorescence light was filtered through a layer of 5% sodium nitrite. A stream of oxygen-free nitrogen was bubbled through the fluorescent solution for 2 mins. before a reading was taken in order to eliminate the quenching effect of atmospheric oxygen (Bowen and Williams, *Trans. Faraday Soc.*, 1939, **35**, 765; Weil-Malherbe and Weiss, *Nature*, 1942, **149**, 471). During the reading, the nitrogen stream was shut off by a tap. The solution to be examined contained up to 3 μg. of benzpyrene in 5 ml. of solvent. For this range of concentration the deflection curve was almost linear. With 1 μg. a deflection of about 65 mm. was obtained. The zero-reading was perfectly stable. A blank, determined with 5 ml. of pure solvent, showed a deflection of 1—2 mm. At least 3 points of a reference curve were established before each series of estimations.

Chromatographic Experiments.—The technique of tapping and compressing generally recommended for the filling of columns (*e.g.*, Zechmeister and von Cholnoky, " Die Chromatographische Adsorptionsmethode," Springer, Wien, 1937) was observed. The rate of filtration was usually 20—60 drops/min. Where volatile solvents were used, filtration was always carried out under pressure to minimise evaporation losses. The progress of the band was watched in the light of the U.V. lamp. As soon as elution had started, fractions of the eluate were collected at frequent intervals, and their volumes recorded.

Adsorption Isotherm Experiments.—These were carried out in sealed test-tubes which were shaken at room temperature for 5—6 hours.

I wish to acknowledge my gratitude to Dr. F. Dickens, who was responsible for the construction of the fluorimeter.

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83. *A New Technique for the Ultimate Micro-analysis of Organic Compounds.*

By Ronald Belcher and Cyril E. Spooner.

In a new technique for the determination of carbon, hydrogen, sulphur, and the halogens in organic compounds on a micro-scale, combustion is effected at 800° by a rapid stream of oxygen (50 ml. per min.) in a tube containing no catalyst or contact material. Carbon and hydrogen are determined gravimetrically, a roll of silver gauze being inserted near the exit end of the combustion tube to absorb the interfering acid gases. Sulphur is quantitatively absorbed and retained as silver sulphate; by extracting this with water the amount of sulphur can also be determined gravimetrically. With compounds containing nitrogen, Elving and McElroy's external absorbents were used to remove nitrogen oxides which would otherwise cause error in the determination of carbon. The sulphur can also be absorbed, and the halogens always are, in external absorbents and determined titrimetrically. Complete absorption occurs at the fast rate of flow used, thus greatly reducing the time for the determinations.

The methods to be described differ from those based on Pregl's micro-methods for the determination of carbon, hydrogen, sulphur, and the halogens in that the combustion is effected by a rapid stream of oxygen in a tube maintained at 800° by means of an electric furnace and containing no catalyst or contact material. The apparatus is similar to that usually adopted in micro-combustion technique.

In the method for the determination of carbon and hydrogen a roll of silver gauze is inserted near the exit end of the combustion tube to absorb interfering gases. If the beak end of the combustion tube is attached by means of a ground joint, the roll of silver gauze may be removed, and the sulphur, if present, can then be determined by estimating the silver sulphate produced.

For the titrimetric determinations of sulphur and the halogens the usual absorbents are used, together with a combustion tube without a catalyst packing. The oxygen flow is much faster than that usually employed in these determinations, but complete absorption occurs. This is in marked contrast to the standard technique, which employs a slower oxygen stream to ensure complete absorption, the combustion often requiring up to one hour as compared with less than 10 mins. with the new methods.

We originated this unpacked tube technique for the ultimate analysis of coal (*Fuel*, 1941, **20**, 130) and, with 0·5 g. of sample, combustion was completed in 10 mins. So far as we are aware this was the first use of an unpacked tube for this purpose, and we have now adapted it to the micro-scale.

For the analysis of organic compounds the conditions are somewhat different from those used for coal: a high temperature is necessary to ensure complete removal of sulphur from the ash in coal, whereas with organic compounds the sulphur can be removed at a much lower temperature unless the substance contains an inorganic element which may form a sulphate only decomposable at a high temperature. The latter type of compound represents a special case and the method has been standardised for the more usual type of compound, a temperature of 800° being used.

Experimental.

Apparatus.—The apparatus is illustrated in the figure, most of the component parts being conventional in micro-chemical laboratories. The oxygen is led from a cylinder or gas-holder to a White–Wright flowmeter adjusted to measure a rate of oxygen flow of 50 ml. per min. From here it passes first through a White–Wright preheater and then through a scavenging train packed with soda asbestos in the first tube and anhydrone in the second; then it is led to the side arm of the combustion tube. All the connections are made with matured rubber tubing, the glass tubes making "butt" joints.

The combustion tube is made of transparent silica of 9 mm. internal diameter and 55 cm. long. It passes through an electric furnace consisting of a lagged silica tube of 15 mm. bore and 25 cm. long with nichrome wire winding and metal body. The furnace is supported on two pillars mounted on an asbestos cement board and is connected in series with a sliding resistance and ammeter. The beak end of the combustion tube protrudes 7·5 cm. out of the furnace, and the inlet portion, which has a 7-cm. length of silica tubing sliding over it, protrudes 22·5 cm.

To calibrate the apparatus, the electric furnace is heated so that the temperature inside the combustion tube measured with a thermocouple is 800°. The current necessary to maintain this temperature is noted, and in all subsequent work is kept at this value. To carry out a blank determination on the assembled apparatus, the oxygen flow is adjusted to 50 ml. per min., the absorption tubes, which are of the standard Pregl type packed with anhydrone and soda asbestos backed with anhydrone, are connected, and finally the Marriotte bottle. After being connected in the apparatus for 10 mins., the absorption tubes should not have increased in weight by more than 0·02 mg.

The fast rate of flow of oxygen obviates the need for a baffle behind the boat, since any tendency to back diffusion is minimised. Also, the fact that no catalyst or contact material is present in the combustion tube simplifies the setting up of the apparatus.

Gravimetric Methods.—Method of combustion when only carbon and hydrogen (and oxygen) are present. To carry out a determination of carbon and hydrogen, weigh 3—5 mg. in a platinum boat, adjust the oxygen flow to 50 ml./min., and attach the weighed anhydrone and soda asbestos absorption tubes. Insert the platinum boat and contents to a distance of 17·5 cm. into the combustion tube, and connect the Marriotte bottle. Carefully decompose the sample by heating the 7-cm. silica tube surrounding the combustion tube with a Bunsen burner, and gradually slide it over that portion of the combustion tube containing the platinum boat, and up to the electric furnace. The whole operation should occupy about 10 mins., whereupon the Marriotte bottle should be disconnected and the absorption tubes detached and weighed.

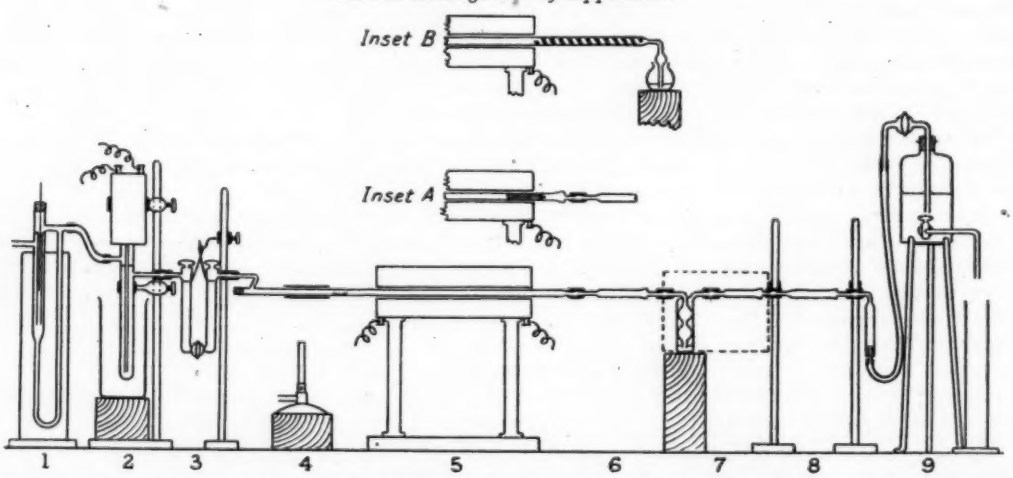
	Calc.		Found.	
Substance.	C, %.	H, %.	C, %.	H, %.
Methylglucoside	43·3	7·21	43·4, 43·4, 43·5, 43·4	7·32, 7·33, 7·24, 7·37
Benzoic acid	68·9	4·95	69·0, 68·9, 68·7	5·15, 4·90, 5·01
Naphthalene	93·7	6·29	93·5, 93·5	6·35, 6·26
Sulphonal	36·8	7·02	36·8, 36·9	6·98, 7·00
Chlorobenzoic acid	53·7	3·19	54·0, 53·7	3·28, 3·22
Chlorophthalic acid	47·9	2·49	47·9	2·55

Some of the results obtained are shown on p. 313, together with results for carbon and hydrogen in some sulphur- and chlorine-containing compounds (*q.v.*): they are comparable in accuracy with those obtained with the general standard methods of micro-analysis.

Method of combustion when sulphur is present. If, in addition to carbon, hydrogen, and oxygen, the substance contains sulphur, this element may be determined simultaneously with the carbon and hydrogen. A modified combustion tube is used having the beak end attached by means of a ground joint. The absorbing material consists of a roll of pure 20-mesh silver gauze, 2½ in. wide, with the edges folded so as to give a width of 2 in., and rolled to fit snugly into the combustion tube (see Inset A). The roll must not be wound too tightly, for only the outer layers will then come in contact with the combustion gases, and the central portion is pulled out at one end to form a grip for crucible tongs. When suitably placed in the combustion tube, this roll absorbs all the oxides of sulphur and the silver sulphate formed can be used to determine the sulphur. The suitable placing is best done by attaching a delivery tube to the exit end of the combustion tube so as to form a bubbler and passing this into a flask containing a few ml. of neutral 1% hydrogen peroxide. Approximately 5 mg. of sulphonal or other compound containing sulphur are then burnt, and the exit gases bubbled through the hydrogen peroxide. When the combustion has been completed, the hydrogen peroxide is titrated with 0·01N-sodium hydroxide, and if any signs of acidity are found the procedure is repeated, the roll of silver gauze being moved ½ in. further into the tube. With the electric furnace used and a roll of silver gauze approximately 2 in. long, it was found that complete absorption of the oxides of sulphur occurred when the outer end of the roll was ½ in. outside the furnace or at any position further inside the furnace.

To carry out the simultaneous determination of carbon, hydrogen, and sulphur, insert the silver gauze roll into the combustion tube to the position previously fixed as most suitable, attach the weighed absorption tubes, and carry out the combustion on 3—5 mg. of substance as described previously. When the combustion is complete, withdraw the roll of silver gauze from the tube, cool it on a metal block, weigh it, and extract it with 200 ml. of boiling water for 2 mins. Wash the roll thoroughly with water, dry it by immersion in alcohol followed by ether, and burn off the residual ether in a

General Arrangement of Apparatus.



1, White–Wright flowmeter. 2, White–Wright preheater. 3, Scavenging train. 4, Bunsen burner for heating the silica tube sliding over that part of the combustion tube containing the sample in a platinum boat. 5, Electric furnace. 6, Water-absorption tube. 7, Arnold bubbler containing N/50-$KMnO_4$ in conc. H_2SO_4 backed with anhydrone for absorbing oxides of nitrogen. 8, Carbon dioxide absorption tube. 9, Marriotte bottle with guard tube.

Inset A. Modification for gravimetric sulphur determination showing position of roll of silver gauze.

Inset B. Modification for titrimetric determinations.

Bunsen flame until the roll begins to glow dull red; cool the roll on the metal block and weigh it. The loss in weight is silver sulphate, and when multiplied by 10·28, it gives the percentage of sulphur in the substance. The determination of carbon and hydrogen is completed as usual.

With compounds containing more than 30% of sulphur, a roll 2 in. long did not absorb all the oxides of sulphur formed, but a roll 3½ in. long gave a satisfactory result for rubeanic acid (see table). The results for sulphur so determined are recorded below for a number of compounds.

Substance.	S, %, calc.	S, %, found.	Substance.	S, %, calc.	S, %, found.
Sulphanilic acid	18·5	18·5, 18·4	Thiocarbanilide	14·1	14·4
Sulphonal	28·1	28·0, 28·1, 28·1	Atropine sulphate	4·7	4·7, 4·9
Cystine	26·7	26·7, 26·8	Rubeanic acid	53·4	54·0

If only the carbon and hydrogen are required, a permanent packing of silver wool can be used in the exit end of the combustion tube (Kirner, *Ind. Eng. Chem. Anal.*, 1935, **7**, 363). In this case a beak-ended tube of the conventional type may be employed.

Method of combustion when halogens are present. For substances containing halogens alone or in addition to sulphur, the silver roll still effects removal of interfering gases, and the same technique is adopted as when sulphur is the only interfering element. The estimation of the weight of silver halide formed cannot be recommended for determination of the halogen present, probably owing to the fact that silver halides tend to volatilise off the silver roll and condense on the cooler parts of the combustion tube. This in no way affects the carbon and hydrogen figures (see, *e.g.*, first table), but it causes the halogen figures to be up to 1% low when determined by loss in weight of the silver roll on extraction in ammonia for silver chloride, and by potassium cyanide solution for the bromide and iodide.

The silver halides formed do not interfere with the gravimetric determination of sulphur as above: the small solubility of the silver halides in the extracting water is depressed beyond interference by the solubility of the silver sulphate.

In this case, however, care is necessary in burning off the residual drying ether so as not to volatilise any silver halide in the flame. This is done by heating the silver gauze roll in the Bunsen flame only until it just begins to glow dull red.

Results for the gravimetric determination of sulphur in the presence of halogens are recorded below: the accuracy of the sulphur determination is unimpaired. Also included are results for the gravimetric determination of sulphur in the presence of oxides of nitrogen: the accuracy of the sulphur figure is again unimpaired because the silver roll is too hot to permit the formation of silver nitrate. Known mixtures of sulphonal with varying amount of chlorobenzoic acid, bromodinitrobenzene, iodoacetic acid, and dinitrobenzoic acid were used in these tests.

Substance.	Radical present.	S, %, calc.	S, %, found.
Sulphonal	Cl	28·1	28·3, 27·7
Sulphonal	Br	28·1	28·0, 28·1
Sulphonal	I	28·1	27·8
7-Iodo-8-hydroxyquinoline-5-sulphonic acid	I	9·12	9·18
Sulphonal	NO_3	28·1	28·1, 27·9, 27·6, 27·9, 27·9

Method of combustion when nitrogen is present. In the case of nitrogenous substances, a two-bulb Arnold bubbler containing either (i) N/50-potassium permanganate in concentrated sulphuric acid or (ii) a saturated solution of potassium dichromate in the same acid, suitably backed with anhydrone, is attached between the anhydrone and the soda asbestos used to absorb water and carbon dioxide. This is to absorb the oxides of nitrogen which may be formed during the combustion and would otherwise cause an error in the carbon figure. The combustion is then carried out in the usual manner. Although it depends on how the nitrogen is combined whether or not oxides of nitrogen are formed, it is probably better to use the bubbler for all compounds containing nitrogen until information is available as to which compounds do and which do not form oxides of nitrogen. In our experiments the bubbler was only necessary for compounds containing nitro- and nitroso-groups.

The usual methods of removing oxides of nitrogen are with a reduced copper spiral or lead peroxide maintained at 180°. Neither of these methods was adopted by us, since the copper spiral would have oxidised rapidly under our conditions and the lead peroxide would have necessitated the introduction of a packing into the combustion tube and a special heating mortar. Elving and McElroy (*Ind. Eng. Chem. Anal.*, 1941, **13**, 660) recommended the use of the above absorbing solution (i) or (ii) in a specially designed bubbler placed between the water and the carbon dioxide absorber. There is a diversity of opinion as to whether or not any absorption of oxides of nitrogen occurs in the water absorber; we have certainly noted an increase in weight of the water absorption tube when using calcium chloride with the Friedrich method. It was claimed by Elving and McElroy, however, that oxides of nitrogen were not absorbed by anhydrone, and our results (below) confirm this. There was negligible increase in weight of the anhydrone tube beyond that required for the hydrogen content, and the carbon figures showed that complete absorption of the oxides of nitrogen by solution (i) was occurring. When the bubbler was not used, the soda asbestos tube increased in weight by an amount corresponding to 4% above that required for the carbon in the one case tested.

	Calc.		Found.	
Substance.	C, %.	H, %.	C, %.	H, %.
Dinitrobenzoic acid	39·6	1·89	40·0, 39·8, 39·8, 39·7, 39·8	2·00, 2·00, 1·90, 1·85, 1·96
Nitroaniline	52·2	4·35	52·5, 52·1	4·44, 4·49
Nitroacetanilide	53·3	4·44	53·5	4·39
Azobenzene	79·1	5·49	78·9	5·64

Titrimetric Methods.—The general set-up of apparatus used in the titrimetric methods for the determination of sulphur and the halogens is the same as that used in the gravimetric methods. A standard spiral combustion tube is used but with a slight modification in that a capillary delivery tube is fused on to the beak end and bent so as to act as a bubbler (see Inset B).

Sulphur. Place in a 50-ml. silica flask 10 ml. of neutral 6% hydrogen peroxide, together with a measured volume of 0·01N-sodium hydroxide, estimated to be slightly in excess of the amount required to neutralise all the sulphuric acid formed. Wet the spiral with the mixture and allow the draining to run back into the flask into which the delivery tube is inserted to act as a bubbler. Adjust the oxygen flow to 50 ml. per min. and insert a 3—5-mg. sample of the compound weighed into a platinum boat. Decompose the sample and complete the combustion by the method previously described. When the tube is cool, wash it out into the flask, make the solution acid with a measured amount of 0·01N-sulphuric acid, boil, and back titrate with 0·01N-sodium hydroxide. Calculate the sulphur from the total acidity produced. The determination may be expedited if a combustion tube is used which allows the glass spiral to be washed without waiting for the furnace to cool, such as that described by Beazley (*Ind. Eng. Chem. Anal.*, 1939, **11**, 229).

If chlorine or bromine is present, the same procedure of absorption in 6% hydrogen peroxide is recommended, followed by the determination of the total acidity. The halogen is determined simultaneously by Gibson aud Caulfield's method (*Analyst*, 1935, **60**, 522), mercuric oxycyanide being used. With compounds which produce oxides of nitrogen, the same authors' barium chromate procedure is recommended.

Chlorine. When chlorine is present alone it is determined by the procedure adopted for sulphur, the chlorine being calculated from the hydrogen chloride absorbed by the alkali.

Iodine. The rapid combustion technique is applied to the Leipert iodine method without further modification. The oxygen flow is maintained at 50 ml./min., and the combustion takes very little longer than 5 mins., but the results show that all the iodine is retained by the spiral wetted with sodium hydroxide. No excess alkali is required in the bubbler when this method is used: if excess is added it causes trouble with the end-point in the subsequent titration.

Some of the results obtained by these methods are recorded below: the accuracy is as good as that given by the general standard methods of titrimetric micro-analysis.

Substance.	Calc., %.	Found, %.	Substance.	Calc., %.	Found, %.
Sulphonal	S, 28·1	27·8, 28·0, 28·3, 27·8, 28·0	Chlorophthalic acid	Cl, 17·7	17·8, 17·9, 17·9, 18·0
Atropine sulphate	S, 4·7	4·6	Iodobenzoic acid	I, 51·2	51·2, 51·1
Sulphanilic acid	S, 18·5	18·8, 18·7, 18·4	Iodoacetic acid	I, 68·3	68·3, 68·2
Chlorobenzoic acid	Cl, 22·7	22·5, 22·5	Iodoform	I, 96·9	96·7

Appendix.—With the method originally developed for the analysis of coal, which used 0·5 g. of sample, an empirical factor of 0·1065 was necessary when converting the silver sulphate into sulphur. The decrease in weight of the silver roll

after extraction was always less than that required by the actual sulphur content of the coal. We were at a loss to account for this, since no sulphur was found to pass the roll. When we applied our technique to the micro-scale we were surprised to note that the correct factor was applicable. Later, in applying the technique to organic compounds on the macro-scale, it was again found that the correct factor gave low results although all the sulphur oxides had been retained by the roll, but the empirical factor worked out for coals when applied gave good results. We now believe this is due to the formation of a small amount of a more complex silver salt, such as silver pyrosulphate, and the mixture of this with the silver sulphate gives rise to the empirical factor 0·1065 as against the theoretical factor for silver sulphate of 0·1028.

The use of an empirical factor for the macro-scale tests was not due to oxides of sulphur passing the silver roll, since by attaching a bubbler containing neutral hydrogen peroxide at the end of the combustion tube negligible acidity was produced. It was not due to the retention of sulphuric acid in the combustion tube, as was shown by washing it out and titrating the washings. It was not due to loss of silver sulphate by the roll, either mechanically or by volatilisation, since the sulphur calculated from the increase in weight as SO_4'' gave the same result as that calculated from the loss in weight as silver sulphate; this would not have been the case had some silver sulphate been lost at the same time as the SO_4 was being absorbed. It was not due to the formation of silver sulphite along with the sulphate since it would have necessitated the formation of two-thirds of the sulphur as silver sulphite and one-third as silver sulphate to satisfy the factor used. Moreover, such an excess of silver sulphite would not have been soluble in the extraction water. It can, therefore, only be due to the formation of some complex sulphur compound, such as silver pyrosulphate, and it would necessitate only 6% of the loss in weight on extraction in water to consist of silver pyrosulphate to account for the empirical factor of 0·1065.

Since silver pyrosulphate, if present, would be hydrolysed by water to give silver hydrogen sulphate, it may be determined in the extraction water by precipitation as barium sulphate. The fact that the whole of the sulphur is retained by the silver and can yield the amount of sulphur ion demanded was shown by two series of experiments.

Direct precipitation by barium chloride cannot be used because silver chloride would be precipitated simultaneously, so in the first series of tests barium nitrate was used. Since it is well known that adsorption occurs with this precipitant, solutions containing equivalent known amounts of sulphate ion and silver ion were precipitated under the same standard conditions as the solutions containing the extracted silver salts. With the comparison solutions the results were always higher than demanded by the amount of sulphate known to be present. This amount was deducted from the figure obtained by precipitating the extraction solution with barium nitrate. The percentage of sulphur calculated from the barium sulphate after deduction of the amount due to adsorption corresponded to that obtained from loss of weight of the silver roll using the empirical factor.

In the second series of tests the silver was precipitated from the extraction water with hydrochloric acid and filtered off. The sulphate ion was then determined in the filtrate by precipitation with barium chloride. In this case also the results obtained by barium sulphate and loss of weight of the silver roll using the empirical factor corresponded.

Although this did not prove the presence of silver pyrosulphate directly, the above experiments indicate that probably some silver salt of a complex sulphur acid is formed which is hydrolysed to sulphate ion. The only one which seems likely to us is the pyrosulphate, and it suggests that in the later stages of the combustion the inner portion of the silver roll already covered with silver sulphate reacts with sulphur trioxide to give this complex compound. On the micro-scale there is probably always sufficient free silver surface to cope with all the oxides of sulphur produced.

Conclusions.—We have shown that our unpacked tube combustion technique can be adapted for the micro-analysis of organic compounds. This technique is simpler and much more rapid than previous methods which rely on a slow stream of oxygen and a tube packed with complex catalysts or oxidants to ensure complete combustion.

We have greatly extended the usefulness of silver gauze in the ultimate analysis of organic compounds, and have shown that it can be used to absorb all the interfering gases except oxides of nitrogen, for which external absorbents are recommended. At the same time we have made it the basis of a gravimetric sulphur determination, thus determining all three elements from a single combustion.

In addition, we have shown that the usual reagents will absorb effectively the combustion products at the fast rate of flow we recommend, thus disproving the idea that in the determination of sulphur and the halogens a slow rate of oxygen flow is necessary. No attempt has been made to find the limiting rate below 50 ml. per min., where quantitative combustion in the same time will occur, since this rate of flow was found to be easy to control.

This technique was first applied to the analysis of coal, and since the chlorine content of coal is usually less than 0·25% for such small amounts the volatility of the silver halide does not prevent the chlorine figure being given to within 0·02%, which is often all that is required.

Since we have already adapted this technique to the macro-analysis of organic compounds, there should be little difficulty in evolving a similar semi-micro technique. Obviously a point will be reached where salts other than silver sulphate are formed, but it should be possible to obtain a suitable factor over a limited range of sulphur contents.

THE UNIVERSITY, SHEFFIELD. [*Received, April 15th,* 1943.]

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84. *New Syntheses of Heterocyclic Compounds. Part II.* 2-*Phenyl*-3 : 4 : 6 : 7-*dibenzo*-1 : 5-*naphthyridine.*

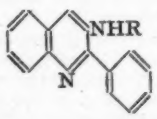
By V. A. PETROW, M. V. STACK, and W. R. WRAGG.

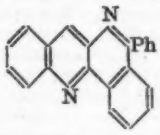
The action of various cyclising agents on the compounds (I)—(IV) has been examined. Ring closure of 3-*benzamido*-2-*phenylquinoline* (VI; R = COPh) to 2-*phenyl*-3 : 4 : 6 : 7-*dibenzo*-1 : 5-*naphthyridine* (VII) has been accomplished by means of phosphoric oxide at 270°.

THE object of the present investigation was the preparation of naphthyridine compounds analogous to the trypanocidal phenanthridinium types of Browning, Morgan, Robb, and Walls (*J. Path. Bact.*, 1938, **46**, 203). The literature on the naphthyridines is limited to some thirty memoirs, and as the methods described therein

proved unsuitable for our purpose, we examined the possibility of applying Pictet and Hubert's phenanthridine synthesis (*Ber.*, 1896, **29**, 1182) to the suitably constituted pyridine and quinoline derivatives (I)—(IV) and (VI).

2- and 3-(o-*Benzamidophenyl*)*pyridine* (I and II) were prepared by benzoylation of the corresponding amino-compounds (Haworth, Heilbron, and Hey, J., 1940, 349). 2-Amino-3-phenylquinoline, obtained from *o*-nitro-α-phenylcinnamonitrile in 30% yield, furnished 2-*acetamido*-3-*phenylquinoline* (III). 2-(o-*Benzamidophenyl*)*quinoline* (IV) was prepared by benzoylation of 2-(*o*-aminophenyl)quinoline (John and Pietsch, *J. pr. Chem.*, 1935, **143**, 245). 3-Amino-2-phenylquinoline (VI; R = H) (*idem*, *ibid.*, 1931, **131**, 346) was conveniently prepared as follows: Benzoylation of phenacylamine hydrochloride in pyridine solution gave the *benzoyl* derivative in excellent yield. Benzoylphenacylamine condensed readily with isatin in alkaline solution to give 3-*benzamido*-2-*phenylquinoline*-4-*carboxylic acid* (V) in 90% yield, and this, heated with syrupy phosphoric acid at 210°, gave (VI; R = H) in 65% yield. The acetyl, *benzoyl*, and p-*nitrobenzoyl* derivatives were prepared.

(VI.) 



(VII.)

Attempts to cyclise (I)—(IV) were not successful. The compounds were recovered unchanged after refluxing with phosphorus oxychloride (cf. Morgan and Walls, J., 1931, 2447). Anhydrous zinc chloride at 300° or syrupy phosphoric acid at 200° led to hydrolysis with regeneration of the amines, an analogous result being obtained by using phosphorus pentachloride–phosphorus oxychloride. Fusion with phosphoric oxide led to resinification. When 3-*benzamido*-2-*phenylquinoline* was heated with phosphoric oxide at 270°, however, facile ring closure occurred to give 2-*phenyl*-3 : 4 : 6 : 7-*dibenzo*-1 : 5-*naphthyridine* in 60% yield.

Experimental.

Microanalyses are by Mr. R. Maxim, University Chemical Laboratories, Cambridge.

2- and 3-(*o*-Nitrophenyl)pyridines (Haworth, Heilbron, and Hey, *loc. cit.*) were reduced in 2 vols. of concentrated hydrochloric acid with 6 parts of stannous chloride in 12 parts of concentrated hydrochloric acid, the reaction being completed by warming for 1 hour on the water-bath. 2-(o-*Aminophenyl*)*pyridine picrate*, m. p. 185—186° (decomp.) (Found: N, 16·4, 16·3. $C_{11}H_{10}N_2,C_6H_3O_7N_3$ requires N, 17·5%), and 3-(o-*aminophenyl*)*pyridine picrate*, m. p. 164° (decomp.) (Found: N, 17·4%), separated from acetone in orange needles and small yellow prisms respectively. The benzoyl derivatives were prepared by treating the amino-compounds, dissolved in pyridine, with 1·2 mols. of benzoyl chloride for 12 hours at room temperature: 2-(o-*Benzamidophenyl*)*pyridine*, needles, m. p. 117°, from absolute alcohol (Found: N, 10·5. $C_{18}H_{14}ON_2$ requires N, 10·2%) [*picrate*, yellow fibrous needles, m. p. 155° (decomp.), from acetone (Found: N, 13·9. $C_{18}H_{14}ON_2,C_6H_3O_7N_3$ requires N, 13·9%)]; 3-(*o-benzamidophenyl*)*pyridine*, squat needles, m. p. 132°, from alcohol–light petroleum (Found: N, 9·7%) [*picrate*, yellow needles, m. p. 168° (decomp.), from acetone (Found: N, 14·1%)].

2-*Amino*-3-*phenylquinoline*.—*o*-Nitro-α-phenylcinnamonitrile (Frost and Meyer, *Annalen*, 1888, **250**, 160) was obtained by adding 4 ml. of sodium ethoxide solution (10 g. of sodium in 159 ml. of alcohol) to 9·16 g. of *o*-nitrobenzaldehyde and 7·0 g. of phenylacetonitrile in 40 ml. of absolute alcohol. After 5 minutes the mixture was refluxed for 1 minute and kept for 12 hours at 0°, and the nitrile collected. Yield 90%, m. p. 129—130°. 1 G. of the nitrile, 50 ml. of spirit, 10 ml. of concentrated hydrochloric acid, and 0·6 g. of tin were refluxed until the tin had dissolved. The mixture was largely diluted with water, tin removed as sulphide, and the filtrate concentrated to small bulk and made alkaline with ammonia. 2-Amino-3-phenylquinoline was precipitated and purified by means of the picrate. Yield, 30%.

2-*Acetamido*-3-*phenylquinoline* was obtained in needles, m. p. 107—108°, from aqueous methyl alcohol (Found: N, 10·8. $C_{17}H_{14}ON_2$ requires N, 10·7%) after treatment of the amino-compound with acetic anhydride (4 vols.) for 30 minutes on the water-bath. 2-(o-*Benzamidophenyl*)*quinoline* was obtained in needles, m. p. 124°, from benzene (Found: C, 81·1; H, 5·0; N, 8·9. $C_{22}H_{16}ON_2$ requires C, 81·5; H, 4·9; N, 8·7%) after 7·6 g. of the amino-compound (John and Pietsch, *J. pr. Chem.*, 1935, **143**, 245) in 25 ml. of pyridine had been treated with 4·8 ml. of benzoyl chloride for 1 hour on the water-bath.

3-*Amino*-2-*phenylquinoline* (VI; R = H).—ω-Bromoacetophenone was prepared as follows (cf. Rather and Reed, *J. Amer. Chem. Soc.*, 1919, **41**, 77): 100 G. of acetophenone, cooled in ice, were shaken with 150 g. of glacial acetic acid and 90 g. of bromine, added in three portions. A portion (10 ml.) was removed, warmed until decolorised, and added to the main bulk; this process was repeated several times and reaction then occurred. The straw-yellow mixture was removed from the ice-bath and, after removal of hydrogen bromide in a current of air, poured on 1 kg. of ice. After crystallising, the bromoacetophenone was collected and dried on a porous plate in a vacuum. Yield, 115 g. It was converted into phenacylphthalimide in 90% yield by Goedeckmeyer's method (*Ber.*, 1888, **21**, 2685), from which phenacylamine hydrochloride was obtained essentially by Gabriel's method (*Ber.*, 1908, **41**, 1132).

12 G. of finely powdered phenacylamine hydrochloride and 21 ml. of dry pyridine were gently warmed. Immediately the suspension has assumed a red colour, 3 ml. of benzoyl chloride were added, solution effected by shaking, and the flask corked and cooled. A further 7 ml. of benzoyl chloride were added with cooling. After 12 hours at room temperature, 50 g. of ice were added with shaking and after 1 hour the precipitated *benzoylphenacylamine* was collected. It formed needles, m. p. 125—126°, from methyl alcohol (Found: C, 75·3; H, 5·7; N, 6·1. $C_{15}H_{13}O_2N$ requires C, 75·3; H, 5·4; N, 5·9%). Unless the above experimental conditions are adhered to, a fraction insoluble in methyl alcohol is obtained from which αγ-diphenylpyrazine, m. p. 193—194° (Found: C, 82·2; H, 5·4; N, 12·3. Calc. for $C_{16}H_{14}N_2$: C, 82·1; H, 6·0; N, 12·0%), and *dibenzoylphenacylamine*, silky white needles, m. p. 173—174°, from methyl alcohol (Found: C, 77·0; H, 5·2; N, 4·3. $C_{22}H_{17}ON_2$ requires C, 77·0; H, 5·0; N, 4·1%), are obtained by fractionation from acetone.

To a warm solution of isatin (7·3 g.) in absolute-alcoholic potassium hydroxide (2·8 g. in 50 ml.), a hot solution of 12 g. of benzoylphenacylamine in 50 ml. of absolute alcohol was added in three portions, with cooling to moderate the reaction. To the clear solution, 18 ml. of potassium hydroxide solution (8·8 g. in 17·5 ml. of water) were added with cooling during 2 minutes. After 3 days at room temperature the mixture was nearly neutralised with hydrochloric acid, the alcohol rapidly boiled off, and 50 ml. of water added towards the end of the heating. After acidification with hydrochloric acid

the mass became solid on stirring. *3-Benzamido-2-phenylquinoline-4-carboxylic acid* (V) formed small, pale yellow needles, m. p. 254—255°, from methyl alcohol (Found : C, 74·5; H, 4·3; N, 7·7. $C_{23}H_{16}O_3N_2$ requires C, 75·0; H, 4·4; N, 7·6%).

5 G. of (V) and 30 ml. of syrupy phosphoric acid (*d* 1·75) were heated in an oil-bath to 170° (sublimation of benzoic acid began) and then, after 30 minutes, slowly to 210°. After 1 hour with occasional stirring the mixture was cooled, diluted with 150 ml. of water, filtered, and made alkaline with ammonia, and the sticky product collected, dissolved in N/10-hydrochloric acid (norit), reprecipitated with ammonia, and dissolved in methyl alcohol. On addition of water to the warm solution a small amount of resinous material separated. The liquid was rapidly filtered, and crystallisation induced by further addition of water. 3-Amino-2-phenylquinoline formed needles, m. p. 121—122°, from light petroleum (Found : C, 81·4; H, 5·5; N, 12·9. Calc. for $C_{15}H_{12}N_2$: C, 81·8; H, 5·5; N, 12·7%). 2 G. of the base in 10 ml. of acetic anhydride were refluxed for 30 minutes and poured on ice; from the sticky green mass, the monoacetyl derivative (VI; R = Ac), m. p. 125°, was obtained by two crystallisations from aqueous methyl alcohol (norit). *3-Benzamido-2-phenylquinoline* (VI; R = COPh), silky needles, m. p. 179—180°, from methyl alcohol (Found : C, 81·2; H, 5·2; N, 8·9. $C_{22}H_{16}ON_2$ requires C, 81·5; H, 4·9; N, 8·6%), was obtained by treating 2·2 g. of the base in pyridine solution with 1·4 ml. of benzoyl chloride for 12 hours at room temperature. *3-p-Nitrobenzamido-2-phenylquinoline*, pale yellow, rhombic crystals, m. p. 223°, from acetone (Found : N, 11·5. $C_{22}H_{15}O_3N_3$ requires N, 11·4%), was obtained by treating 2·2 g. of the base in 7 ml. of pyridine with 1·86 g. of *p*-nitrobenzoyl chloride for 1 hour on the water-bath and precipitating the product with dilute hydrochloric acid.

2-Phenyl-3 : 4 : 6 : 7-dibenzo-1 : 5-naphthyridine (VII).—An intimate mixture of 3-benzamido-2-phenylquinoline (2 g.) with excess of phosphoric oxide was heated for 2 hours at 270—280° (calcium chloride tube), and water and ice added; on stirring, a greenish-black solid separated. It was collected, extracted with ammonia–acetone (norit), and the extract concentrated until crystallisation took place. *2-Phenyl-3 : 4 : 6 : 7-dibenzo-1 : 5-naphthyridine* formed silky needles, m. p. 197—198°, from aqueous methyl alcohol (Found : C, 86·1; H, 4·7; N, 9·3. $C_{22}H_{14}N_2$ requires C, 86·3; H, 4·7; N, 9·2%). The *monopicrate* formed yellow plates, m. p. 240—241°, from acetone (Found : N, 13·3. $C_{22}H_{14}N_2,C_6H_3O_7N_3$ requires N, 13·1%).

Attempts to cyclise the acetyl derivative (VI; R = Ac) gave a *product*, m. p. 199° (Found : C, 81·7; H, 5·2; N, 11·4%), which could not be identified. The *p*-nitrobenzoyl derivative (VI; R = $CO{\cdot}C_6H_4{\cdot}NO_2$) underwent resinification on similar treatment with phosphoric oxide.

The authors are indebted to I.C.I. (Dyestuffs) Ltd. for grants and gifts of materials. The facilities kindly placed at the disposal of Queen Mary College by the University of Cambridge are gratefully acknowledged.

QUEEN MARY COLLEGE (UNIVERSITY OF LONDON),
c/o THE UNIVERSITY CHEMICAL LABORATORIES, CAMBRIDGE. [*Received, March 3rd*, 1943.]

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85. *The Monoreduction of Dinitronaphthalenes in Acid Solution and of* 1 : 5- *and* 1 : 6-*Dinitronaphthalene by Aqueous Sodium Sulphide.*

By HERBERT H. HODGSON and HAROLD S. TURNER.

Improvements in the methods for the monoreduction of dinitronaphthalenes in acid and in alkaline environment are described.

A SOLUTION of stannous chloride in glacial acetic acid–hydrogen chloride can be advantageously used for the monoreduction of the α-nitro-group in αβ-dinitronaphthalenes. 1 : 6-Dinitronaphthalene is thus much more efficiently reduced to 6-nitro-1-naphthylamine than by Věsely and Dvorák's process (*Bull. Soc. chim.*, 1923, **33**, 319). From 1 : 3-dinitronaphthalene, a mixture of 3-nitro-1- and 4-nitro-2-naphthylamine is obtained, the former greatly predominating (cf. Věsely and Dvorák, *loc. cit.*). 1 : 5- and 1 : 8-Dinitronaphthalenes, however, are reduced to the corresponding diamines whether an excess or a deficit of the reducing agent is used. Aqueous sodium sulphide reduces 1 : 6-dinitronaphthalene to 5-nitro-2-naphthylamine and 1 : 5-dinitronaphthalene to 5-nitro-1-naphthylamine.

EXPERIMENTAL.

Preparation of the Reducing Agent.—A current of hydrogen chloride was passed through stannous chloride crystals (64 g.) in hot glacial acetic acid (140 c.c.) and continued while the clear solution was cooled to 0°. This then contained the correct amount of reducing agent for 20 g. of a dinitronaphthalene.

Monoreduction of 1 : 6-*Dinitronaphthalene.*—A solution of the compound (20 g.) in hot glacial acetic acid (160 c.c.) was rapidly chilled, and the fine suspension treated during 45 minutes below 30° with the reducing agent; stirring was continued for 2 hours, and the mixture kept overnight. The pasty product was mixed with water (20 c.c.), heated to 60—70°, and distilled at 65° under reduced pressure to remove most of the acetic acid, the 6-nitro-1-naphthylamine stannichloride washed out with water (150 c.c.) and basified below 25° with 20% aqueous sodium hydroxide (450—550 c.c.), and the orange-red mixture of 6-nitro-1-naphthylamine and unchanged 1 : 6-dinitronaphthalene washed with water and dried in a vacuum. The small quantity of 1 : 6-naphthylenediamine also formed remained in the filtrate from the basification mixture and was extracted therefrom by chloroform. The orange-red precipitate was worked up either (*a*) by three extractions (300, 100, and 50 c.c.) with boiling ether, and passage of hydrogen chloride through the extract to precipitate 6-nitro-1-naphthylamine hydrochloride (10·5—11 g.), the unchanged 1 : 6-dinitronaphthalene (6 g.) being recovered from the ether-insoluble residue and the filtrate; or (*b*) by repeated extraction with boiling 10% hydrochloric acid until the extract gave no precipitate on addition of ammonia, the combined extracts being then basified with ammonia at 0°. Yield of 6-nitro-1-naphthylamine by (*a*) or (*b*), *ca.* 7·2 g. (*ca.* 60%); it crystallised from chloroform in bright orange-red needles, and from aqueous alcohol in deep red needles, m. p. 167·5° (Found : N, 15·0. Calc. : N, 14·9%).

Reduction of 1 : 3-*Dinitronaphthalene.*—The crude basified mixture of 3-nitro-1- and 4-nitro-2-naphthylamine obtained from 1 : 3-dinitronaphthalene (20 g.) was ether-extracted (500 c.c.), and on passage of hydrogen chloride the extract afforded a precipitate of mixed hydrochlorides (11·9 g.); the filtrate therefrom yielded 1 : 3-dinitronaphthalene

(4·1 g.). After basification, the mixed nitroamines (7·6 g.) were dissolved in hot hydrochloric acid (50 c.c. of acid, *d* 1·16, and 320 c.c. of water), the solution boiled with charcoal for a few minutes and filtered, the filtrate chilled in ice, and the crystalline precipitate of 3-nitro-1-naphthylamine hydrochloride removed and basified to give almost pure 3-nitro-1-naphthylamine (5·85 g., m. p. 131°), which had m. p. 137° after crystallisation from dilute alcohol. The acid filtrate on basification yielded the remainder of the mixed amines (1·75 g., m. p. 75°).

Improved Monoreduction of 1 : 5-*Dinitronaphthalene by Sodium Sulphide.*—Finely powdered 1 : 5-dinitronaphthalene (25 g.), wetted with alcohol (10 c.c.) (it tends to float on water), was stirred with hot water (450 c.c.), and then treated at 95° during 15 minutes with a solution of crystallised sodium sulphide (27·5 g.) in water (50 c.c.) (Hodgson and Walker, J., 1933, 1346). The water-washed residue of 5-nitro-1-naphthylamine was repeatedly extracted with hot 7% hydrochloric acid (total, 3 l.), and the extract basified at 0° with ammonia to give 5-nitro-1-naphthylamine (13 g., 60·5% yield; Hodgson and Walker, *loc. cit.*, give 36%), which crystallised from ligroin in red needles, m. p. 119°.

Monoreduction of 1 : 6-*Dinitronaphthalene by Sodium Sulphide.*—1 : 6-Dinitronaphthalene (3 g.), suspended in a boiling mixture of alcohol (20 c.c.) and water (15 c.c.), was treated with a solution of crystallised sodium sulphide (5·25 g.; 1·6 g.-mol. per g.-mol. of nitro-compound) in the minimum amount of water; after 30 minutes' refluxing, 5-nitro-2-naphthylamine (1·7 g.) separated on cooling; it crystallised from hot aqueous alcohol in small red needles, m. p. and mixed m. p. with an authentic specimen, 146° (Hodgson and Crook, J., 1936, 1844, give m. p. 145—146°) (Found : N, 15·0. Calc. : N, 14·9%).

The authors thank I.C.I. (Dyestuffs) Ltd. for gifts of chemicals.

TECHNICAL COLLEGE, HUDDERSFIELD. [*Received, December 19th*, 1942.]

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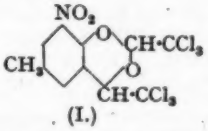
86. *The Action of Alkalis upon Substituted Benzdioxins.*

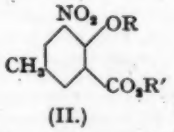
By H. IRVING and (in part) E. G. CURTIS.

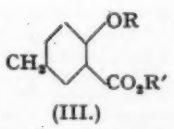
Chloral has been condensed with 2- and with 3-nitro-*p*-cresol. The action of alcoholic potash upon the 7- and the 8-*nitro*-6-*methyl*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin* (IV and I) so formed, upon the corresponding amines, and upon other representatives of this heterocyclic system has been examined and found to confirm the reaction mechanisms previously put forward.

IN a previous paper (J., 1934, 325) it was shown that the action of alcoholic potash in opening the hetero-ring of various derivatives of 1 : 3-benzdioxin (as I) was initiated by an attack by an ethoxyl ion at the 9-carbon atom, provided that this had been rendered sufficiently kationoid by the presence of enough, and suitably oriented, electron-attracting substituents (*e.g.*, CCl_3 groups) in positions 2 and 4 of the hetero-ring and others (*e.g.*, NO_2, CO_2H) in the 6- and/or 8-positions of the benzene ring.

8-*Nitro*-6-*methyl*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin* (I) is now found to resemble the isomeric 6-nitro-7-methyl-2 : 4-bistrichloromethyl-1 : 3-benzdioxin (Chattaway and Calvet, J., 1928, 1089), for when it was boiled with alcoholic potash the intrusion of an ethoxyl group opened the hetero-ring and 5-*nitro*-4-*ethoxy*-m-*toluic acid* (II; R = Et, R′ = H) was obtained, whose structure was confirmed by synthesis from 4-hydroxy-*m*-toluic acid (III; R,R′ = H) through ethyl 4-ethoxy-*m*-toluate (III; R,R′ = Et) and 4-*ethoxy*-m-*toluic acid* (III; R = Et, R′ = H).

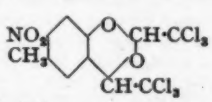

(I.)


(II.)


(III.)

An unsuccessful attempt was made to synthesise the acid (II; R = Et, R′ = H) by another route in which the nitration of 4-hydroxy-*m*-toluic acid constituted the first stage. The mononitrated acid, hitherto unoriented, was shown to be 5-nitro-4-hydroxy-*m*-toluic acid (II; R,R′ = H) by decarboxylation to 3-nitro-*p*-cresol.

All the benzdioxins hitherto prepared whose hetero-rings have proved susceptible to alkaline fission have contained electron-attracting substituents in positions 6 and/or 8. In 7-*nitro*-6-*methyl*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin* (IV), however, the nitro-group is situated meta to the 9-carbon atom, whence its electron-attracting effect cannot now be relayed tautomerically. It was expected that its general effect in making this 9-carbon atom feebly kationoid would be too slight to promote attack by anionoid reagents and consequent ring fission, and, in fact, when (IV) was boiled with alcoholic potash, the hetero-ring remained unbroken and 7-*nitro*-6-*methyl*-2 : 4-*bisdichloromethylene*-1 : 3-*benzdioxin* (V) was formed.

(IV.)  (V.)

The stability conferred on the hetero-ring by an amino-group in the benzene ring (Chattaway and Calvet, *loc. cit.*) is further illustrated by the action of alcoholic potash upon 8-*amino*-6-*methyl*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin* (as I), which yields 8-*amino*-6-*methyl*-2 : 3-*bisdichloromethylene*-1 : 3-*benzdioxin* (as V). A methoxyl group is equally effective, for, when treated with alcoholic potash, the hetero-ring of 5 : 7-*dichloro*-6-*methoxy*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin* remains intact, loss of two molecules of hydrogen chloride

yielding 5 : 7-*dichloro*-6-*methoxy*-2 : 4-*bisdichloromethylene*-1 : 3-*benzdioxin*, which was also obtained by methylating the unsaturated dioxin previously described (Chattaway and Calvet, *loc. cit.*). Similar stability is conferred by an azo-group, for 6-*p*-tolueneazo-2 : 4-bistrichloromethyl-1 : 3-benzdioxin yields 6-p-*tolueneazo*-2 : 4-*bisdichloromethylene*-1 : 3-*benzdioxin* when treated with alcoholic potash.

In more complex derivatives of benzdioxin the critical rôle of the 9-carbon atom in determining the stability of the hetero-ring is illustrated by the action of alcoholic potash of various strengths upon *cis*- and *trans*-di-(2 : 4-bistrichloromethyl-1 : 3-benzdioxinyl)-6 : 6′-sulphone, where sulphonyl and trichloromethyl are the activating groups (Chattaway and Bell, J., 1934, 45). Similarly it is now found that, though di-(6-nitrobenzdioxinyl)-8 : 8′-methane (Chattaway and Goepp, J., 1933, 699) resembles 6-nitrobenzdioxin itself in its stability towards alkali (Chattaway and Irving, *loc. cit.*), the combined effect of nitro- and carbonyl groups in di-(6-nitrobenzdioxinyl)-8 : 8′ ketone renders the hetero-ring so prone to fission that with boiling 5% aqueous caustic soda it yields formaldehyde and 5 : 5′-*dinitro*-2 : 2′-*dihydroxy*-3 : 3′-*bishydroxymethylbenzophenone*, characterised by a *phenylhydrazone*.

Experimental.

8-*Nitro*-6-*methyl*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin* (I).—3-Nitro-*p*-cresol (10 g.; 1 mol.) and chloral hydrate (21·6 g.; 2 mols.) were dissolved in concentrated sulphuric acid (100 c.c.) and shaken vigorously at intervals for 18 hours. The product was poured on ice and washed with water and then with dilute aqueous ammonia, to remove unchanged material. After drying at 100°, the benzdioxin was recrystallised several times from boiling alcohol (solubility, about 20 g./l.) and then from glacial acetic acid, from which 8-*nitro*-6-*methyl*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin* (I) separated (21·5 g.; 75%) in short, colourless prisms, m. p. 175—176° (Found : Cl, 49·4. $C_{11}H_7O_4NCl_6$ requires Cl, 49·5%).

Action of alkali. (*a*) The benzdioxin (I) was unaffected by refluxing for 6 hours with excess of 2·5% alcoholic potassium acetate.

(*b*) Potassium hydroxide (10 g.; 8 mols.) in ethyl alcohol (25 c.c.) was added to a solution of the dioxin (10 g.; 1 mol.) in 100 c.c. of alcohol, whereupon a red colour developed and chloroform was liberated. The reaction was completed by gentle heating on the water-bath for 5 minutes; boiling water (200 c.c.) was then added, the solution made slightly acid with hydrochloric acid, and the alcohol removed in a rapid current of steam. From the cooled red solution so obtained, ether extracted a viscous mass of brown crystals, which were purified by solution in very dilute, hot aqueous alkali and, after boiling with animal charcoal, reprecipitated by strong acidification with hydrochloric acid. Repetition of this process several times gave 5-*nitro*-4-*ethoxy*-m-*toluic acid* (II; R = Et, R′ = H), m. p. 142°. After repeated crystallisation from light petroleum (b. p. 100—120°), in which it was sparingly soluble, it separated in slender needles, m. p. 148—149° (Found : C, 53·4; H, 4·6; N, 6·25. $C_{10}H_{11}O_5N$ requires C, 53·3; H, 4·9; N, 6·2%), which slowly developed a yellow colour on exposure to the air but gave no colour reaction with ferric chloride.

Synthesis of 4-*Ethoxy*-m-*toluic Acid* (III; R = Et, R′ = H).—Ethyl 4-ethoxy-*m*-toluate (III; R,R′ = Et), prepared by Auwers's method (*J. pr. Chem.*, 1924, **107**, 355) from ethyl 4-hydroxy-*m*-toluate, ethyl bromide, and sodium ethoxide, or by refluxing for 10 hours a suspension of 4-hydroxy-*m*-toluic acid (10 g.), ethyl sulphate (30 g.), potassium carbonate (20 g.), and dry acetone (50 c.c.), had b. p. 144—145°/10 mm. Saponification with a slight excess of boiling aqueous alcoholic potash (2 hours) gave 4-*ethoxy*-m-*toluic acid* (III; R = Et, R′ = H) in almost colourless needles, m. p. 76—78° after crystallisation from aqueous alcohol or benzene. It gave no marked colour with ferric chloride (Found : C, 66·4; H, 6·6. $C_{10}H_{12}O_3$ requires C, 66·7; H, 6·7%).

Nitration was effected by dropwise addition of nitric acid (3·3 g., *d* 1·40) to a solution of the ethoxy-acid (5 g.) in acetic acid (15 c.c.) below 5° (1 hour). 5-Nitro-4-ethoxy-*m*-toluic acid, which separated when the mixture was poured on ice, was purified as described above, forming slender needles, m. p. 148—149° alone or mixed with the specimen described above.

Decarboxylation of 5-*Nitro*-4-*hydroxy*-m-*toluic Acid* (II; R = R′ = H).—The nitro-acid, prepared by the controlled nitration of 4-hydroxy-*m*-toluic acid (Einhorn and Pfyl, *Annalen*, 1900, **311**, 51), separated from boiling benzene–acetone in colourless, friable prisms, m. p. 176—177° (Kostanecki and Niementowski, *Ber.*, 1885, **18**, 254, give m. p. 172°). The position of the nitro-group was established by refluxing the acid (3·5 g.) with quinoline (20 c.c.) for 5 hours. From the cold reaction mixture, aqueous potash extracted 3-nitro-*p*-cresol, m. p. and mixed m. p. 35—36°. Further confirmation of the position of the nitro-group is afforded by the observation that 3 : 5-dinitro-*p*-cresol is the principal product obtained when 4-hydroxy-*m*-toluic acid is rapidly treated with concentrated nitric acid at or above room temperature.

7-*Nitro*-6-*methyl*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin* (IV).—This was prepared from 2-nitro-*p*-cresol (63 g.; 1 mol.), chloral hydrate (140·5 g.; 2 mols.), and 850 c.c. of concentrated sulphuric acid. After 14 days the *benzdioxin*, isolated and purified as in the case of (I), was obtained in friable, colourless prisms, m. p. 143° (Found : Cl, 49·5%).

Action of alcoholic potash. A solution of the benzdioxin (IV) (1·4 g., 1 mol.) in alcohol (75 c.c.) was mixed with a solution of potassium hydroxide (3 g.; 16·5 mols.) in alcohol (40 c.c.) and boiled for ½ hour; potassium chloride separated. After filtration, addition of water yielded 7-*nitro*-6-*methyl*-2 : 4-*bisdichloromethylene*-1 : 3-*benzdioxin* (V), which crystallised from alcohol, in which it was very soluble, in colourless needles, m. p. 101°, which gradually turned red and decomposed in the air (Found : Cl, 39·5. $C_{11}H_5O_4NCl_4$ requires Cl, 39·7%).

8-*Amino*-6-*methyl*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin* (as I).—The nitro-compound (I) (10·3 g.) was warmed on the water-bath (½ hour) with stannous chloride (55 g.) in acetic acid (250 c.c.) and acetic anhydride (50 g.) saturated with hydrogen chloride (cf. Albert and Linnell, J., 1936, 1615). After cooling and filtration, addition of water gave 8-*amino*-6-*methyl*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin* as a flocculent white precipitate (7·8 g., 80%), which, after being collected and dried, crystallised from alcohol, in which it was readily soluble, in compact colourless prisms, m. p. 140° (Found : Cl, 53·7. $C_{11}H_9O_2NCl_6$ requires Cl, 53·2%). The *acetyl* derivative crystallised from alcohol, in which it was very soluble, in highly refracting, colourless prisms, m. p. 171·5° (Found : Cl, 48·3. $C_{13}H_{11}O_3NCl_6$ requires Cl, 48·2%).

Action of alcoholic potash. The amino-compound (3 g.) in alcohol (30 c.c.) was boiled with caustic potash (3 g.; 7 mols.) in alcohol (50 c.c.); potassium chloride separated. After ½ hour, when much water was added to the cold filtrate, 8-*amino*-6-*methyl*-2 : 4-*bisdichloromethylene*-1 : 3-*benzdioxin* separated. It crystallised from alcohol as a felt of colourless needles, m. p. 121° (Found : Cl, 44·0. $C_{11}H_7O_2NCl_4$ requires Cl, 43·4%). The *acetyl* derivative crystallised from alcohol in fine, long, colourless needles, m. p. 201° (Found : Cl, 38·3. $C_{13}H_9O_3NCl_4$ requires Cl, 38·4%).

Methylation of 5 : 7-*Dichloro*-6-*hydroxy*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin*.—The benzdioxin (15·2 g.; 1 mol.) was shaken with water (10 c.c.) and methyl sulphate (10 c.c.; 2½ mols.), and dilute aqueous potash, added in portions. 5 : 7-*Dichloro*-6-*methoxy*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin* separated as a dirty white, granular material (15 g.). After being dried, it crystallised from boiling acetic acid, in which it was moderately easily soluble, in clusters of fine colourless rhombs, m. p. 189—190° (Found : Cl, 60·7. $C_{11}H_6O_2Cl_8$ requires Cl, 60·4%).

5 : 7-*Dichloro*-6-*methoxy*-2 : 4-*bisdichloromethylene*-1 : 3-*benzdioxin*, similarly obtained from 5 : 7-dichloro-6-hydroxy-

2 : 4-bisdichloromethylene-1 : 3-benzdioxin (Chattaway and Calvet, J., 1928, 2913), was very soluble in acetic acid and separated thence or from boiling alcohol, in which it was much less soluble, in colourless prisms, m. p. 114·5—115° (Found : Cl, 54·0. $C_{11}H_4O_2Cl_6$ requires Cl, 53·8%). The same compound was obtained when the preceding methoxy-benzdioxin (11·3 g.; 1 mol.) was boiled for 15 minutes with a solution of caustic potash (12·2 g.; 9 mols.) in alcohol (120 c.c.).

Action of Alcoholic Potash upon 6-p-*Toluneazo*-2 : 4-*bistrichloromethyl*-1 : 3-*benzdioxin*.—The azo-compound (4 g.; 1 mol.) (Chattaway and Calvet, *loc. cit.*) was refluxed (½ hour) with caustic potash (4·5 g.; 9 mols.) in alcohol (50 c.c.). A rapid current of steam removed the alcohol and precipitated 6-p-*toluneazo*-2 : 4-*bisdichloromethylene*-1 : 3-*benzdioxin* (2·8 g.). This, crystallised from alcohol, in which it was moderately easily soluble, and then from acetic acid, in which it was readily soluble, formed clumps of very small, compact, orange prisms, m. p. 147—148° (Found : Cl, 34·0. $C_{17}H_{10}O_2N_2Cl_4$ requires Cl, 34·1%).

Hydrolysis of Di-(6-*nitrobenzdioxinyl*)-8 : 8′ *Ketone*.—The ketone (5 g.) was boiled with 50 c.c. of 5% caustic soda solution for 1 hour; formaldehyde was evolved. After cooling and filtration, the red solution deposited 5 : 5′-*dinitro*-2 : 2′-*dihydroxy*-3 : 3′-*bishydroxymethylbenzophenone*, which separated from dilute aqueous alcohol as a felt of tiny, colourless needles, m. p. 260° (Found : N, 7·9. $C_{15}H_{12}O_9N_2$ requires N, 7·7%). The *phenylhydrazone* separated from alcohol, in which it was very sparingly soluble, in compact, deep red rhombs, m. p. 226—227° (Found : N, 12·2. $C_{21}H_{18}O_8N_4$ requires N, 12·3%).

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87. *Preparation of Diazo-naphthols and Nitration of* 4-*Bromoaceto*-1-*naphthalide.*

By HERBERT H. HODGSON and STANLEY BIRTWELL.

Four methods are described for the preparation of diazo-naphthols (internal diazo-oxides), one of which exemplifies the facilitating action of copper salts for the removal of nitro-groups. The nitration of 4-bromoaceto-1-naphthalide is inhibited by urea. Chromoisomerism is exhibited by 4-chloro- and 4-bromo-2-nitro-1-naphthylamine.

THE diazotisation of weak bases such as 2 : 4-dinitro-1-naphthylamine in nitrosylsulphuric acid solution only takes place when a weak acid such as acetic or phosphoric acid is added (Misslin, *Helv. Chim. Acta*, 1920, **3**, 626; Schoutissen, *J. Amer. Chem. Soc.*, 1933, **55**, 4531; Hodgson and Walker, J., 1933, 1620), indicating that diazotisation and elimination of the nitro-group take place simultaneously when water is added to the mixture. The two processes have now been separated. If glacial acetic acid alone is added, only diazotisation takes place, the diazo-oxide being precipitated on subsequent dilution (Method I). If, however, cupric carbonate is added to the acetic acid (Method II), diazo-oxide formation begins at once, though dilution with water is necessary to obtain the full yield. The excess of nitrous acid used by Morgan and Evens (J., 1919, **115**, 1126) therefore acts instead of the copper carbonate as an oxidising agent, in the absence of which only diazotisation occurs (compare the formation of 1 : 4 : 6-trichloro-3-bromobenzene in the Sandmeyer reaction on 3-bromo-4 : 6-dinitro-1-benzenediazonium sulphate; Hodgson, *J. Soc. Dyers and Col.*, 1926, **42**, 365). Partial neutralisation of the sulphuric acid solution with ammonia to remove the sulphuric acid alone, may be necessary for the isolation of the diazo-oxide (Method III).

Diazo-oxide formation also takes place in glacial acetic acid by the action of sodium nitrite alone at moderate temperatures (Method IV), to be ascertained separately for each individual amine, and previous procedures (Meldola and Eyre, J., 1902, **81**, 988; Orton, *Proc.*, 1902, **18**, 12; Hodgson and Foster, J., 1942, 30) have been modified accordingly; *e.g.*, 4-*iodonaphthalene*-1 : 2-*diazo-oxide* could only be prepared satisfactorily by Method IV.

Addition of excess of urea to the nitric acid used in the nitration of 4-bromoaceto-1-naphthalide (Morgan and Godden, J., 1910, **97**, 1709) should be avoided, as it inhibits the nitration.

The hydrolysis of the 4-halogeno-2-nitroaceto-1-naphthalides by 50% sulphuric acid and ethyl alcohol requires at least 8 hours for completion, but is completed in 90 minutes in a boiling solution of concentrated hydrochloric and glacial acetic acids.

4-Chloro- and 4-bromo-2-nitro-1-naphthylamines exhibit chromoisomerism, crystallising from aqueous pyridine or chlorobenzene in orange-brown needles, and from anhydrous pyridine in yellow needles, which can be changed into the brown variety.

EXPERIMENTAL.

Nitration of 4-*Chloroaceto*-1-*naphthalide*.—4-Chloroaceto-1-naphthalide prepared by addition of sodium chlorate (12 g.) in water (50 c.c.) to a stirred suspension of aceto-1-naphthalide (37 g.) in glacial acetic acid (120 c.c.) and hydrochloric acid (46 c.c., *d* 1·16) below 40° (yield, 28·5 g.; 65%) was suitable for nitration; it crystallised from alcohol in colourless needles, m. p. 187° (Reverdin and Crépieux, *Ber.*, 1900, **33**, 682, give m. p. 186·5°). A suspension of the naphthalide (85 g.) in glacial acetic acid (450 c.c.) was treated with nitric acid (40 c.c., *d* 1·42) at room temperature, and the mixture heated to 40°; reaction was complete in a few minutes at 50—55°. The 4-chloro-2-nitroaceto-1-naphthalide was filtered off (55 g.) from the almost solid mass and washed with glacial acetic acid (50 c.c.) and with cold water. The mother-liquors afforded another 28 g. (total yield, 81%). The material crystallised from alcohol in pale yellow needles, m.p. 220° (Hodgson and Elliott, J., 1935, 1850, give m. p. 219°) (Found : Cl, 13·3 Calc. : Cl, 13·4%).

Nitration of 4-*Bromoaceto*-1-*naphthalide with and without Urea*.—4-Bromoaceto-1-naphthalide was obtained in 95% yield when a solution of aceto-1-naphthalide (56 g.) in glacial acetic acid (360 c.c.) was treated at 18—20° with bromine (48 g.) in glacial acetic acid (192 c.c. of a 25% solution); it was collected (75 g.) after 30 minutes, washed with glacial acetic acid (50 c.c.) and with water, and was then suitable for nitration. It crystallised from alcohol in colourless needles, m. p. 193° (Meldola, *Ber.*, 1878, **11**, 1906, gives m. p. 193°).

4-Bromoaceto-1-naphthalide (75 g.), suspended in glacial acetic acid (320 c.c.), was treated at room temperature with nitric acid (30 c.c., *d* 1·42); reaction began at 50° and was completed at 60—65°. The 4-bromo-2-nitroaceto-1-naphthalide

obtained (70 g.; 79% yield), after being washed with glacial acetic acid (100 c.c.) and with cold water, crystallised from alcohol in pale yellow needles, m. p. 231—233° (Morgan and Godden, *loc. cit.*, give m. p. 230°) (Found: Br, 25·8. Calc.: Br, 25·9%).

In a similar experiment in which the nitrating solution contained urea the m. p. of the product indicated that nitration was very incomplete.

Hydrolysis of 4-Halogeno-2-nitroaceto-1-naphthalides.—The naphthalide (25 g.) was refluxed with hydrochloric acid (125 c.c., *d* 1·16) and glacial acetic acid (375 c.c.) for 90 minutes. The amine crystallised on cooling; the total yield was obtained by dilution with water. 4-Chloro-2-nitro-1-naphthylamine (20·2 g.; 96% yield) separated from aqueous pyridine or chlorobenzene in orange-brown needles, m. p. 205° (Hodgson and Elliott, *loc. cit.*, give m. p. 202°) (Found: Cl, 15·7. Calc.: Cl, 15·9%). 4-Bromo-2-nitro-1-naphthylamine (20·2 g.; 93% yield) crystallised from either solvent in orange-brown needles, m. p. 200° (Morgan and Godden, *loc. cit.*, give m. p. 200°) (Found: Br, 29·8. Calc.: Br, 29·9%), and from anhydrous pyridine in bright yellow needles, m. p. 197° (Found: Br, 29·8%).

Preparation of Internal Diazo-oxides (Diazo-naphthols).—Method I (compare Morgan and Evens, *loc. cit.*). 2 : 4-Dinitro-1-naphthylamine (46·6 g.) was gradually added to sulphuric acid (200 c.c., *d* 1·84) below 10°, followed by sodium nitrite (14·0 g.) in sulphuric acid (100 c.c., *d* 1·84) at such a rate that the temperature did not rise above 10°. The mixture was added to ice-cooled glacial acetic acid (300 c.c.) below 20°, stirred for 30 minutes, and run into water (2 l.) containing ice (1 kg.) during 30 minutes. The precipitated orange-yellow diazo-oxide was filtered off, washed three times with cold water (5 l.), and dried at *ca.* 50°. Yield, 41 g. (95%); m. p. 130—133° (decomp.).

Method II. 2 : 4-Dinitro-1-naphthylamine (7 g.), dissolved in sulphuric acid (30 c.c., *d* 1·84), was treated as above with a solution of sodium nitrite (2·2 g.) in sulphuric acid (15 c.c., *d* 1·84), and the mixture added below 25° to a solution of copper carbonate (15 g.) in glacial acetic acid (120 c.c.). Rapid precipitation of 4-nitronaphthalene-1 : 2-diazo-oxide occurred, which was completed by dilution with water. Yield, 6·1 g. (95%).

Method III. A solution of 4-bromo-2-nitro-1-naphthylamine (26·7 g.) in hot glacial acetic acid (200 c.c.) was chilled rapidly, and the paste added between 10° and 20° to a solution of sodium nitrite (8 g.) in sulphuric acid (56 c.c., *d* 1·84), 25 c.c. of glacial acetic acid being used during manipulation. After 15 minutes' stirring, a mixture of ice and water (300 c.c.) was added, the temperature rising to *ca.* 20°. After removal of a brown precipitate the filtrate was diluted with more ice and water (400 g.) and then, during 1 hour, neutralised in part below 20° by the addition of aqueous ammonia (120 c.c., *d* 0·880) in water (120 c.c.), which is the amount required to neutralise the sulphuric but not the acetic acid. When the addition was not too rapid, 4-bromonaphthalene-1 : 2-diazo-oxide separated as a heavy, orange, granular precipitate (22 g., 90% yield), which was air-dried in the dark; m. p. 120°. Too rapid addition precipitated the compound as a tar, which, however, was slowly transformed into yellow needles with evolution of gas. This method is also admirable for the preparation of 4-chloronaphthalene-1 : 2-diazo-oxide, which is obtained in 90% yield as an orange-yellow compound, m.p. 130°. It is not convenient to prepare 4-iodonaphthalene-1 : 2-diazo-oxide by this procedure, since tar formation during the partial neutralisation could not be prevented, however slowly the ammonia was added.

Method IV (cf. Meldola and Eyre, *loc. cit.*). 4-Iodo-2-nitro-1-naphthylamine (6·3 g.) (Hodgson and Elliott, *loc. cit.*) was suspended in glacial acetic acid (40 c.c.) and stirred at *ca.* 30° for 30 minutes with sodium nitrite (1·6 g.; slight excess); the mixture was then heated to 40—45°, and sodium nitrite (0·2 g.) added to complete the diazotisation, denoted by complete dissolution of the amine. (This further addition was unnecessary in the preparation of 4-chloro- and 4-bromo-naphthalene-1 : 2-diazo-oxides.) The solution was filtered, cooled, and diluted with ice and water, whereby *4-iodonaphthalene-1 : 2-diazo-oxide* was precipitated (5·6 g.; 95% yield), which crystallised from boiling water (very sparingly soluble in the cold) in felted yellow needles, m. p. 142° (decomp.) (Found: N, 9·6; I, 42·7. $C_{10}H_5ON_2I$ requires N, 9·5; I, 42·9%).

4-Chloronaphthalene-1 : 2-diazo-oxide, prepared in like manner, but at 25—30° throughout (95% yield), crystallised from boiling water in pale yellow, feathery needles, m. p. 138° (decomp.) (Found: N, 6·9; Cl, 17·2. $C_{10}H_5ON_2Cl$ requires N, 6·8; Cl, 17·3%).

4-Bromonaphthalene-1 : 2-diazo-oxide, similarly prepared in 96% yield (initially at room temperature, finally at 40° to complete the dissolution of the amine), crystallised from hot water in yellow needles, m. p. 133° (decomp.) (Orton, *Proc.*, 1902, **18**, 252, gives m. p. 132—133°) (Found: Br, 32·0. Calc.: Br, 32·1%).

Preparation of 4-Nitronaphthalene-1 : 2-diazo-oxide by Method IV.—2 : 4-Dinitro-1-naphthylamine (4·7 g.), suspended in glacial acetic acid (100 c.c.), was stirred for several hours with sodium nitrite (2 g.) at room temperature, but no diazo-oxide appeared to be formed. When the temperature was gradually raised to 90°, and more sodium nitrite (2—3 g.) added, dissolution to a deep red solution occurred. After filtration, cooling, and dilution with ice-water, 4-nitronaphthalene-1 : 2-diazo-oxide was precipitated as a bright yellow powder (3·5 g.; 81·5% yield), m. p. 120° (decomp.).

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88. *Bacterial Inhibition by Metabolite Analogues. Part V. Reactions and Antibacterial Properties of* p-*Diazine Di*-N-*oxides.*

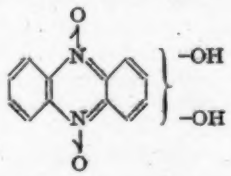
By HENRY MCILWAIN.

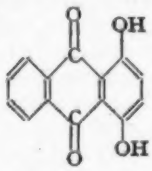
The bacterial pigment iodinin has been regenerated from the product of its reduction, and its structure as a dihydroxyphenazine di-*N*-oxide confirmed. A number of quinoxaline di-*N*-oxides have been prepared for examination as antibacterial agents, for reasons outlined below, and they exhibited the following properties. The oxides retained the basic characters of the diazines, but those with saturated 2-substituents had also acidic properties which could be explained as due to tautomeric oxime forms. *2-Methylquinoxaline di*-N-*oxide* changed in alkali to a rich blue product, and the conversion was greatly accelerated by light. Two quinoxaline di-*N*-oxides characterised by unsubstituted 2-positions reacted with keto-methylene compounds in dilute alkaline solutions. All the di-*N*-oxides were readily reduced to the diazines, and quinoxalines, but not phenazines, underwent further fission by zinc in acid solution to *o*-phenylenediamine and monoketones.

All the di-*N*-oxides were inhibitory to bacterial growth in concentrations in which their parent diazines were inactive.

THE pigment of *Chromobacterium iodinum*, here termed iodinin, was considered by analysis, degradation, and comparison with related compounds to be the di-*N*-oxide of a dihydroxyphenazine (I) (Clemo and McIlwain,

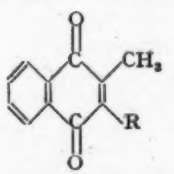
J., 1938, 479). In concentrations of less than 1 μg. per ml. it inhibited growth of some pathogenic bacteria (McIlwain, *Nature*, **1941**, **148**, 628); the inhibition was relatively little influenced by extracts of many natural materials, but was antagonised by some containing naphthaquinone and anthraquinone derivatives, and by certain pure hydroxyanthraquinones, *e.g.*, by quinizarin (II) (McIlwain, *Biochem. J.*, in press). The mutual interaction of (I) and (II) may be by competition for common receptor sites of the affected organism, as there

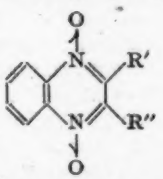
(I.) 

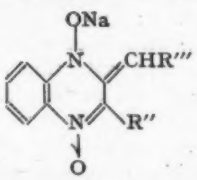
(II.) 

is a structural and polar similarity between the two compounds analogous to that existing between other molecules which interact as bacterial inhibitors and their antagonists, for example, between sulphanilamide and *p*-aminobenzoic acid. The latter relationship has been successfully used in designing further inhibitory compounds corresponding to carboxylic acids other than *p*-aminobenzoic acid (McIlwain, *Lancet*, **1942**, **1**, 412). This subject has been pursued in the present investigation, which is of compounds related to antihæmorrhagic substances in the manner in which iodinin is related to the antagonistic anthraquinones.

2-Methyl-1 : 4-naphthaquinone (III, R = H) promotes growth of Johne's bacillus (Woolley and McCarter, *Proc. Soc. exp. Biol., N.Y.*, **1940**, **45**, 357); unknown compounds of antihæmorrhagic activity occur in other bacteria (Almquist, Pentler, and Mecchi, *ibid.*, **1938**, **38**, 336) and phthiocol (III, R = OH) in *M. tuberculosis*. If these materials are of functional value to the organisms producing them, related quinoxaline di-*N*-oxides

(III.) 

(IV.) 



(V.)

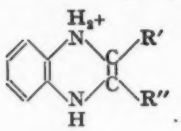
may be inhibitory. Previous descriptions of such oxides have not been found, and the following representatives have now been prepared. 2-Methylquinoxaline was obtained from *iso*nitrosoacetone and *o*-phenylenediamine (Bennett and Willis, J., 1928, 1966), and 2-methyl-3-*n*-amylquinoxaline by a similar reaction with *iso*nitrosomethyl *n*-hexyl ketone. These bases, and also quinoxaline and 1 : 2 : 3 : 4-tetrahydrophenazine (2 : 3-*cyclo*tetramethylenequinoxaline), were converted into their di-*N*-oxides by hydrogen peroxide in acetic acid solution. Iodinin was regenerated from the dihydroxyphenazine produced on reduction, by perbenzoic acid in benzene. 2-*Methylquinoxaline di*-N-*oxide* (IV; R′ = CH_3, R″ = H) is isosteric with the antihæmorrhagic 2-methylnaphthaquinone, and the *oxide* of 2-methyl-3-*n*-amylquinoxaline (IV; R′ = CH_3, R″ = $[CH_2]_4{\cdot}CH_3$) approximates to similar relationship with the K vitamins.

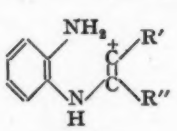
The diazine di-*N*-oxides were basic, forming slightly soluble phosphotungstates in 2N-sulphuric acid, but no precipitate with lead, silver, or mercuric salts. The substituted quinoxaline oxides showed also acidic properties which were probably due to their tautomeric forms (V); thus, while sodium hydroxide facilitated extraction of phenazine di-*N*-oxide from aqueous solution by ether or chloroform, it hindered such extraction of 2-methylquinoxaline, 2-methyl-3-*n*-amylquinoxaline, and of 1 : 2 : 3 : 4-tetrahydrophenazine oxides. Examples of tautomerism which, as (IV, R′ = CH_2R''') $\rightleftharpoons$ (V), involve saturated 2-substituents of *N*-substituted quinoxalines have previously been observed in quaternary quinoxaline salts (McIlwain, J., 1937, 1701). These cases also were associated with a valency change of the 1-N atom, and again analogous reactions did not occur with phenazine derivatives. Secondary changes took place subsequently in alkaline solutions of 2-methylquinoxaline and 1 : 2 : 3 : 4-tetrahydrophenazine di-*N*-oxides. A reaction peculiar to the former oxide was its conversion in alkaline solution into a rich blue product. The change was accelerated by illumination, and could be seen proceeding in sunlight in a few seconds. A much slower photochemical change in 2-methylquinoxaline itself was observed by Böttcher (*Ber.*, **1913**, **46**, 3085). Rapid photochemical reactions have previously been encountered in phenazonium salts (McIlwain, J., 1937, 1704); the isosteric 2-methyl-1 : 4-naphthaquinone is also photosensitive and the K-vitamins are also sensitive to alkali.

The nature of the product of the photochemical change has not been established, nor that of the secondary change in 1 : 2 : 3 : 4-tetrahydrophenazine di-*N*-oxide, which was not noticeably accelerated by light. Further indication of the reactivity of the *N*-oxides was obtained through reactions with ketomethylene compounds. The di-*N*-oxides of quinoxaline and its 2-methyl derivative reacted in very dilute alkaline solutions with acetone, producing pronounced brown products. As the oxides of 2-methyl-3-*n*-amylquinoxaline, phenazine, and its 1 : 2 : 3 : 4-tetrahydro-derivative did not so react, the change probably involves a hydrogen atom of the 2- or 3-position.

All the diazine di-*N*-oxides investigated liberated iodine from acid solutions of potassium iodide and were reduced by neutral or slightly acid sodium hydrosulphite to the parent diazines. The quinoxaline oxides were peculiar in undergoing more extensive degradation with zinc in acid solutions, yielding, in addition to the

1 : 2 : 3 : 4-tetrahydro-compound, *o*-phenylenediamine and a monoketone or aldehyde (IV ⟶ IX). This was not primarily a reaction of the *N*-oxides [and is thus not analogous to rearrangements of certain *N*-oxides to ketones in acids observed by Haworth and Perkin (J., 1926, 445, 1769)], but was given also by the diazines themselves. It was a reaction of quinoxalines as distinct from phenazines, which under similar conditions yielded their *NN'*-dihydro-compounds. 1 : 2 : 3 : 4-Tetrahydrophenazine gave a mixture of 1 : 2 : 3 : 4 : 9 : 10 : 11 : 12-octahydrophenazines, *o*-phenylenediamine, and *cyclo*hexanone. The reaction is not a hydrolysis of 1 : 2 : 3 : 4-tetrahydroquinoxalines (the products of alkaline reduction), which were found stable to acid; nor is it analogous to Emde's reaction or to the fission of benziminazole salts to alkylated *o*-phenylenediamines, which occur in alkaline solution. In dihydrophenazines, as distinct from dihydroquinoxalines, the 2 : 3-double bond does not exist as such, forming part of an aromatic system; also, alkylated *o*-phenylenediamines can form dihydrochlorides, while dihydrophenazine forms only a monohydrochloride. Both these points increase the mesomeric forms possible to the diazine ring in dihydroquinoxaline cations. One such form is (VII), in which the tendency


(VI.)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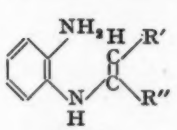
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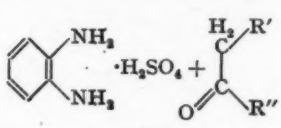
(IX.)

of N^+ to acquire electrons is satisfied by rupture of the 1 : 2-C–N bond; the energy for this breakage may be supplied by resonance, further salt formation at the amino-groups formed, and supply of electrons and a proton to the 2-C atom to give the Schiff's base (VIII), capable in the acid reaction solution of further fission to a monoketone and amine salt (IX). Electrons supplied immediately to the form (VI) would constitute the normal reduction to a 1 : 2 : 3 : 4-tetrahydroquinoxaline, occurring simultaneously in acid solution. In alkali, forms such as (VII) would not exist and some of the associated processes, such as salt formation with the product, would not occur; reduction to the 1 : 2 : 3 : 4-tetrahydro-compound only can then be understood.

All the di-*N*-oxides were found to inhibit growth of *Streptococcus hæmolyticus*; the parent bases in comparable or higher concentrations were usually indifferent. *C. diphtheriæ* also was inhibited by the compounds investigated. The concentrations required for bacteriostasis were in all cases greater than those required of iodinin; the compounds have, however, not yet been tested with organisms requiring pre-formed K-vitamins for growth.

Experimental.

p-*Diazine Di*-N-*oxides*.—The method of Clemo and McIlwain (J., 1938, 479) can be advantageously carried out with less reagents, the procedure here employed being: the base (1/200 mol.) in glacial acetic acid (20 ml.) with hydrogen peroxide (5 ml. of 100 vol.) was heated at 50° for 16 hours. Separation of the products is described individually below.

The quinoxaline oxides were yellow or brown, and the phenazine oxides orange or red, crystalline solids, melting (with decomposition and evolution of gas) higher than the bases from which they were derived. Heating just below 100°, alone or in solution, also usually caused slow decomposition. The oxides liberated iodine from iodides; satisfactory conditions for discriminating between this reaction and the liberation of iodine which is caused by certain diazines themselves (especially phenazines, by their being reduced to their *NN'*-dihydro-compounds) were: the oxide (1—2 mg.) in 50% aqueous acetic acid (0·5 ml.) with potassium iodide (0·25 ml. of 10% aqueous solution) was heated at 100° for 5 minutes, diluted with water, and starch solution added. Degradative reduction was carried out as follows: the oxide or quinoxaline (0·5 g.) in 5N-sulphuric acid (20 ml.) with excess of zinc, after a few minutes at room temperature smelt of the ketone or aldehyde, and the reaction was completed by warming. The colour changed through yellow-green (of the semi-quinone) to paler yellow; the aldehyde or ketone was separated by aspiration or ether-extraction of the acid solution, and *o*-phenylenediamine obtained by ether extraction of the basified aqueous layer. In this way acetaldehyde- and *cyclo*hexanone-2 : 4-dinitrophenylhydrazones and *o*-phenylenediamine were isolated from quinoxaline and 1 : 2 : 3 : 4-tetrahydrophenazine and from their di-*N*-oxides, and keto-compounds and *o*-phenylenediamines detected from 2-methyl- and 2-methyl-3-*n*-amyl-quinoxaline di-*N*-oxides. No phenolic or ketonic material was detected in similar reductions of phenazine or phenazine di-*N*-oxide, nor from reductions of them in more or in less acid solutions.

Quinoxaline Di-N-*oxide*.—Quinoxaline (b. p. 106—107°/20 mm., m. p. 33°; from *o*-phenylenediamine and glyoxal sodium bisulphite) was oxidised, ice and excess of 10N-sodium hydroxide added, and the oxide extracted by shaking four times with equal volumes of chloroform. The dried (sodium sulphate) extract was evaporated, and the *product* which crystallised collected in two batches and recrystallised from water (16 ml.), forming bright yellow needles, which were dried at 60°. Yield 55%, m. p. 238—239° to a yellow liquid which almost immediately darkened and evolved gas (Found: N, 16·9. $C_8H_6O_2N_2$ requires N, 17·3%).

Colourless dilute solutions of the oxide (*e.g.*, of 4 mg./ml.) in N-sodium hydroxide produced with acetone (20 mg./ml.) a yellow colour deepening in a few minutes to brown and forming also a light-coloured flocculent precipitate. The reaction was given to varying degrees by acetophenone, ethyl acetoacetate and oxaloacetate, by sodium pyruvate and α-ketoglutarate but not by benzaldehyde, acetate, butyrate, glycine or glucose. Alkaline hyrogen peroxide caused no comparable reaction with the ketomethylene compounds; the oxide itself became brown only in more concentrated (5N) alkali.

2-*Methylquinoxaline Di*-N-*oxide*.—The quinoxaline (b. p. 124—125°/20 mm., from *o*-phenylenediamine and *iso*nitrosoacetone in 40—50% yield; see below) was oxidised, ice added, the mixture neutralised with 10N-sodium hydroxide (excess of alkali caused decomposition and hindered extraction) and extracted six times with equal volumes of chloroform. The dried extracts were evaporated to 5 ml., light petroleum (30 ml.) added, the precipitation repeated with the evaporated mother-liquors, and the combined precipitates crystallised from benzene; yield 65%; m. p. 180—181° (Found: C, 61·3; H, 4·5; N, 16·0. $C_9H_8O_2N_2$ requires C, 61·4; H, 4·5; N, 15·9%).

Dilute alkaline solutions of the oxide, which were almost colourless (*e.g.*, of 2 mg./ml.) gradually became strongly green or blue, and a Prussian-blue-like amorphous material separated from them. The change was more rapid in N-than in N/10-sodium hydroxide, and was much accelerated by sunlight. The blue product was apparently complex:

it was insoluble in chloroform, ether, and in 2N-acid or alkali, and on reduction in neutral solution by hydrosulphite did not yield a volatile base. The oxide resembled that of quinoxaline in its reaction with acetone.

2-Methyl-3-n-amylquinoxaline.—*iso*Nitrosomethyl *n*-hexyl ketone was prepared according to Behr-Bregowski (*Ber.*, 1897, **30**, 1515) and recrystallised from light petroleum by ice-cooling. To a mixture of water (20 ml.), glacial acetic acid (6 ml.), and concentrated hydrochloric acid (3 ml.), *o*-phenylenediamine (7·2 g.) and the *iso*nitroso-compound (10·9 g.) were added and shaken; the mixture became warm and by further heating was refluxed for 3 minutes. An oily layer formed and was separated by extracting the cooled mixture with light petroleum, leaving the aqueous layer (a). The extract was filtered from a solid (b) which separated, washed successively with a little 0·5N-sulphuric acid (c) and 2N-sodium hydroxide (d), dried, and evaporated; the product distilled in a vacuum (175°/12 mm.) as a very pale yellow liquid (6·0 g.) solidifying on cooling. It was recrystallised from light petroleum, forming rosettes of stout colourless prisms, m. p. 46°, which were very slightly soluble in water.

Various modifications in procedure failed to afford a higher yield. Solid (b) separated also from the reaction mixture (a) on neutralisation and in all weighed 3·2 g.; the crude substance, m. p. 160°, on heating in acid liberated methyl hexyl ketone and on reduction in acid yielded *o*-phenylenediamine. It was considered a product of condensation of 2 mols. of the *iso*nitrosoketone with one of *o*-phenylenediamine, as a corresponding excess (2·9 g.) of the diamine was found in (a) and (c) but little or no *iso*nitroso-ketone or other compound could be recovered from (d). Qualitative evidence was obtained of a similar side reaction in the preparation of 2-methylquinoxaline, of which the yield was similarly low.

2-Methyl-3-n-amylquinoxaline Di-N-oxide.—The quinoxaline was oxidised for 40 hours, ice added, and the mixture neutralised with 10N-sodium hydroxide; the product, which separated as an oil that solidified on scratching, was filtered off, washed with water, and dried in a vacuum. It crystallised from methanol or aqueous methanol, with ice-cooling, in yellow needles (58%) of the *oxide*, m. p. 107° (Found: C, 67·9; H, 7·4; N, 11·65. $C_{14}H_{18}O_2N_2$ requires C, 68·3; H, 7·3; N, 11·4%). The oxide was more soluble in water than was the parent base, and with strong alkali gave a yellow solution from which it was not extracted by ether.

1 : 2 : 3 : 4-Tetrahydrophenazine Di-N-oxide.—The base (Clemo and McIlwain, J., 1934, 1991) was oxidised to a brown solution, which was neutralised with ice-cooling. A buff-coloured granular precipitate separated and was filtered off, washed with water, and dried in a vacuum. It partly decomposed on crystallising from water, but was obtained in brown-yellow needles from alcohol or benzene; m. p. on rapid heating, 188° (Found: N, 13·2. $C_{12}H_{12}O_2N_2$ requires N, 12·95%). Alkaline solutions of the *oxide*, initially brown, changed slowly through orange to red-purple; the change was accelerated by heating but little if at all by light.

Phenazine Di-N-oxide.—Prepared by the general method, most of the oxide (cf. Clemo and McIlwain, J., 1938, 479) crystallised from the mixture and was separated in 90—95% yield by dilution with water, cooling and filtration. Oxidation by perbenzoic acid was also attempted to act as model in the preparation of iodinin; the same compound, m. p. 204°, was obtained in 60% yield.

Iodinin.—The general method of *N*-oxide formation was not found applicable to iodinin. Following Meisenheimer (*Ber.*, 1926, **59**, 1848) and Puschkareva (*J. Gen. Chem. Russia*, 1938, **8**, 151, 158), perbenzoic acid was found successful. The acid was prepared in chloroform solution, and assayed according to Tiffeneau (*Org. Synth., Coll. Vol.*, 1932, **1**, 422) and was transferred to benzene by repeated addition of the solvent and partial evaporation under reduced pressure.

The dihydroxyphenazine (15 mg.) derived (Clemo and McIlwain, J., 1938, 479) from the pigment, in benzene (10 ml.), was kept at room temperature with a benzene solution of perbenzoic acid calculated to contain 4 equivs. of available oxygen. The solution gradually became red (but similar ones in chloroform did not); aliquots diluted 20 to 50 times in benzene were compared colorimetrically with a standard solution of iodinin and showed a 40% conversion in 1 day, 55% after 3 days, and no further increase after 5 days. The bulk was then added to a column of activated alumina prepared with benzene; brown-yellow material was strongly adsorbed and the red product formed a wide band below it. The chromatogram was developed with benzene; the yellow material remained at the top of the column while the red band moved rapidly down and changed to blue. A benzene solution (red) of iodinin was adsorbed on alumina as a blue band, and the transitory red appearance of the adsorbate of the reaction mixture was probably due to the initial presence of much benzoic acid, which was partly removed by the solvent first passing through the column. The blue band from the reaction mixture was also washed through the column, the resulting red solution evaporated to dryness, benzoic acid extracted with a little ether, and the pigment crystallised from benzene, yielding purple needles (3 mg.) with a bronze metallic glint; m. p. alone or with iodinin, 236°.

Bacteriological Testing.—Aqueous solutions of the *N*-oxides were sterilised by filtration, and different quantities added to media, which were then inoculated and examined for growth during the following 7 days. General methods are described elsewhere (McIlwain, *Biochem. J.*, in press); the present tests used three strains of *Streptococcus hæmolyticus* (the Richards, another Group A, and a Group G organism) grown in the peptone medium *b* (*loc. cit.*) and three of *C. diphtheriæ* (one *gravis* and two *mitis* strains) grown in a casein hydrolysate medium (McIlwain, *Brit. J. exp. Path.*, 1942, **23**, 95). The concentrations needed for different degrees of bacteriostasis are shown in the Table. When strains reacted differently, a range of values is given.

Antibacterial Effects of N-Oxides.

Organism.	Base or *N*-oxide.	Concentration (*M*) of base or *N*-oxide causing— no inhibition.	prevention of visible growth for (days): 1.	2—4.	5 or more.
Strep. hæm.	Iodinin	10^{-7}	5×10^{-7}	10^{-6}	$1{\cdot}5—2 \times 10^{-6}$
C. diphtheriæ	,,	10^{-7}	$10^{-7}—10^{-6}$	$10^{-6}—10^{-5}$	10^{-5}
Strep. hæm.	Phenazine di-*N*-oxide	4×10^{-6}	$2—4 \times 10^{-5}$	$4—8 \times 10^{-5}$	$6 \times 10^{-5}—10^{-4}$
,, ,,	1 : 2 : 3 : 4-Tetrahydrophenazine	10^{-5}	—	—	—
,, ,,	,, ,, ,, ,, di-*N*-oxide	4×10^{-5}	2×10^{-4}	$5 \times 10^{-4}—10^{-3}$	10^{-3}
C. diphtheriæ	1 : 2 : 3 : 4-Tetrahydrophenazine	10^{-5}	—	—	—
,, ,,	,, ,, ,, ,, di-*N*-oxide	4×10^{-5}	$4 \times 10^{-5}—2 \times 10^{-4}$	$2 \times 10^{-4}—10^{-3}$	10^{-3}
Strep. hæm.	2-Methyl-3-*n*-amylquinoxaline	5×10^{-4}	—	—	—
,, ,,	,, ,, ,, ,, di-*N*-oxide	2×10^{-4}	5×10^{-4}	—	—
C. diphtheriæ	2-Methyl-3-*n*-amylquinoxaline	5×10^{-4}	—	—	—
,, ,,	,, ,, ,, ,, di-*N*-oxide	10^{-4}	2×10^{-4}	10^{-3}	—
Strep. hæm.	2-Methylquinoxaline	$5 \times 10^{-4}—2 \times 10^{-3}$	2×10^{-3}	—	—
,, ,,	,, ,, ,, di-*N*-oxide	$2 \times 10^{-4}—2 \times 10^{-4}$	$2 \times 10^{-4}—10^{-3}$	—	$5 \times 10^{-4}—10^{-3}$

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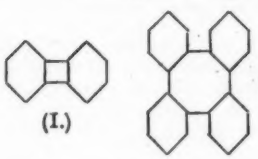
89. *Benzcyclooctatetraenes. Part III. Diphenylene and Tetraphenylene.*

By W. S. Rapson, R. G. Shuttleworth, and (in part †) J. N. van Niekerk.

The action of cupric chloride on the Grignard reagent prepared from 2 : 2′-dibromodiphenyl has yielded a compound, $C_{12}H_8$, identical with that formulated as diphenylene (I) by Lothrop (*J. Amer. Chem. Soc.*, 1941, **63**, 1187), and a compound, $C_{24}H_{16}$, formulated as 1 : 2 : 3 : 4 : 5 : 6 : 7 : 8-*tetrabenz*-$\Delta^{1:3:5:7}$-cyclo*octatetraene* (*tetraphenylene*) (II). Crystallographic data are presented for the latter and the preparation of a *monobromo*- and a *tetranitro*-derivative is described.

The application of the Krizewsky–Turner reaction to 2 : 2′-dibromodiphenyl was stated (J., 1941, 488) to yield diphenyl and traces of a halogen-free, picrate-forming substance, m. p. 107°. Repetition of the work on a larger scale has shown the presence among the products of a hydrocarbon, $C_{24}H_{16}$, and also that the material mentioned above is a hydrocarbon, $C_{12}H_8$, identical in physical characteristics with that since described by Lothrop (*J. Amer. Chem. Soc.*, 1941, **63**, 1187) and formulated by him as being diphenylene (I) (compare, however, Baker, *Nature*, 1942, **150**, 210).

The compound $C_{24}H_{16}$ is almost certainly *tetraphenylene* (II). It is a colourless, high-melting, exceedingly stable compound, being recovered unchanged after treatment with potassium permanganate in boiling acetone. It failed to yield addition compounds with trinitrobenzene, picric acid, and styphnic acid, but crystallised from some solvents in rather stable, solvated forms. A *tetranitro*-derivative was prepared, and bromination to yield a *monobromo*-derivative occurred only in the presence of a catalyst. Crystallographic data for the hydrocarbon are presented and its full crystal structure is under investigation.



(I.) (II.)

In some of our experiments widely varying quantities of diphenyl and 2 : 2′-diphenyldiphenyl accompanied diphenylene and tetraphenylene. Their formation was apparently due to reactions catalysed by traces of water, since neither could be isolated from the reaction mixture when moisture was rigorously excluded.

Experimental.

Action of Cupric Chloride on the Grignard Reagent from 2 : 2′-Dibromodiphenyl.—Reaction between 2 : 2′-dibromodiphenyl (78 g.; 1 mol.) and magnesium (7·3 g.; 1·2 mols.) in dry ether (500 c.c.) was initiated with the aid of iodine; after 48 hours' refluxing, a white precipitate formed. The heavy red oil which separated on the gradual addition, with ice-cooling and stirring, of dry cupric chloride (78 g.; 2·16 mols.) changed to a reddish-brown precipitate after 3—4 hours' refluxing. The ethereal solution was decanted, and the precipitate treated with ether and water, residual magnesium then reacting. The cuprous chloride was dissolved by the addition of hydrochloric acid, and the combined ethereal solutions filtered, washed with concentrated hydrochloric acid and with water, dried, and evaporated. The residue was separated into fractions (1) b. p. 105—130°/4 mm., (2) b. p. 130—170°/4 mm., and (3) b. p. 170—250°/4 mm., leaving a resinous residue. Fractions (1) and (2) were refractionated through a short column, fractions boiling at 105°/4 mm. (95°/0·2 mm.) and 150°/4 mm. (135°/0·2 mm.) respectively being collected. Fraction (1) was dissolved in its own volume of absolute alcohol; light yellow needles of diphenylene (1·0 g.) separated on ice-cooling, m. p. 111—112° after recrystallisation from alcohol (Lothrop, *loc. cit.*, gives m. p. 109—110°) (Found: C, 94·7; H, 5·4. Calc. for $C_{12}H_8$: C, 94·7; H, 5·3%). To the hot mother-liquor was added solid picric acid (1 g. per 10 c.c.), the solution cooled in ice, and the picrate filtered off. Further small portions of picric acid were introduced, and the above process repeated until picric acid itself separated. The picrate, recrystallised once from the minimum quantity (2 c.c. per g. of picrate) of absolute alcohol saturated with picric acid (ice-cooling) and once from absolute alcohol, formed red needles (1·4 g.), m. p. 121—122°, and on decomposition with dilute aqueous ammonia yielded a further 0·5 g. of diphenylene (total yield, 4%). Fraction (2) on recrystallisation from alcohol gave recovered 2 : 2′-dibromodiphenyl (7—10 g.). Fraction (3) on sublimation at 200°/0·2 mm. or on treatment with an equal volume of light petroleum (b. p. 80—90°) yielded almost pure tetraphenylene (6·0 g.; 16%), m. p. 230°, and 233° after recrystallisation.

If complete exclusion of moisture from the reactants had not been achieved, diphenyl and 2 : 2′-diphenyldiphenyl accompanied the other products. Thus in one experiment 6 g. of diphenyl and 0·4 g. of diphenylene were obtained from fraction (1), and 3·7 g. of tetraphenylene and 3·0 g. of 2 : 2′-diphenyldiphenyl from fraction (3). An attempt to bring about a chromatographic separation of diphenyl and diphenylene having failed, fraction (1) was in such cases treated with its own volume of absolute alcohol and cooled in ice; diphenyl separated and was filtered off. Treatment of the mother-liquor with picric acid in the manner detailed above then yielded diphenylene picrate. When 2 : 2′-diphenyldiphenyl accompanied tetraphenylene in fraction (3), the latter separated first on treatment of the distillate with light petroleum. Redistillation of the residue then yielded a fraction, b. p. 205—215°/1 mm., which deposited crystals on treatment with an equal volume of light petroleum, followed by ice-cooling. Purification from absolute alcohol gave large, colourless, brittle prisms, m. p. 118—119°, not depressed by authentic 2 : 2′-diphenyldiphenyl (Bowden, J., 1931, 1111).

† *Crystallography and Space Group of Tetraphenylene.*—The crystals used for the *X*-ray examination were transparent and colourless, with well-developed faces, making accurate goniometric measurements possible. The symmetry is monoclinic holohedral as judged from external form. Single crystal oscillation photographs made with Fe-$K\alpha$ radiation show that the structure may be based on a unit cell with $a = 16{\cdot}39$, $b = 13{\cdot}16$, $c = 15{\cdot}60$ A., $\beta = 100°\ 40'$. An inspection of the general *X*-ray reflections reveals that this cell is not primitive, but centred on the A face. The space group is determined uniquely as $C2h^6$, or A2/a in the Hermann–Maugin notation.

The density of the crystal, determined by the flotation method, lies between 1·20 and 1·25 g./c.c. The unit cell thus contains eight molecules of tetraphenylene, and the true density is 1·214 g./c.c.

The external form exhibited by the crystals is more conveniently described crystallographically in terms of axes with a monoclinic angle $\beta = 123°\ 56'$, and axial ratios $a : b : c = 1{\cdot}475 : 1 : 1{\cdot}185$. The eight faces of the form {111} referred to these axes are commonly developed, together with the (100) and (001) faces, and very rarely the (010) faces. Structurally, however, the unit cell based on these axes is less convenient than the one given above.

Tetraphenylene showed a relatively low solubility in most solvents. It crystallised without solvation from ethyl alcohol, *n*-butyl alcohol, acetic acid, ethyl acetate, methyl ethyl ketone, or nitrobenzene [Found: C, 94·5, 94·75; H, 5·25, 5·6; *M* (Rast), 300, 306. $C_{24}H_{16}$ requires C, 94·7; H, 5·3%; *M*, 304], but gave solvated forms in transparent rectangular prisms from the following solvents:

Solvent.	Analysis of solvate. C.	H.	Loss on heating.*	
C_6H_6	94·7, 94·5	5·5, 5·6	11·0% at 110°	$2C_{24}H_{16}.C_6H_6$ requires C, 94·5; H, 5·5; loss, 11·1%.
CCl_4	77·2	4·4	—	$2C_{24}H_{16}.CCl_4$ requires C, 77·2; H, 4·2%.
Dioxan	89·65	5·95	—	$2C_{24}H_{16}.C_4H_8O_2$ requires C, 89·65; H, 5·75%.
C_5H_5N	—	—	11·5% at 150°	$2C_{24}H_{16}.C_5H_5N$ requires loss, 11·5%.
$CHCl_3$	—	—	16·5% at 150°	$2C_{24}H_{16}.CHCl_3$ requires loss, 16·4%.
$COMe_2$	—	—	8·65% at 150°	$2C_{24}H_{16}.C_3H_6O$ requires loss, 8·7%.

* Blank estimations were made to allow for pronounced sublimation of tetraphenylene.

x-*Bromotetraphenylene*.—Tetraphenylene (0·6 g.) was mixed with carbon tetrachloride (20 c.c.), bromine (0·48 g.) in carbon tetrachloride (10 c.c.) added, together with a little powdered iron, and the mixture heated till bromine vapour had disappeared. After filtration and evaporation, the residue was boiled in ethyl acetate with active carbon, and the solution filtered and treated with absolute alcohol; a white powder (0·78 g.), m. p. 130—140°, separated. A long series of crystallisations from ethyl acetate–ethyl alcohol gave x-*bromotetraphenylene*, m. p. 182°, free from tetraphenylene (Found: C, 75·5; H, 4·1. $C_{24}H_{15}Br$ requires C, 75·2; H, 3·9%).

Tetranitrotetraphenylene.—When tetraphenylene (0·4 g.; 1 mol.), dissolved in cold carbon tetrachloride (10 c.c.), was shaken with nitric acid (*d* 1·5, 0·4 g.; 4 mols.) and concentrated sulphuric acid (0·8 g.) in a closed tube, a semi-solid product immediately formed. When this had solidified, a little water was added, and the solid (0·7 g.) collected. It liquefied in contact with most solvents except alcohol and carbon tetrachloride, in both of which it was only very slightly soluble. It was taken up in benzene and filtered, the solvent allowed to evaporate until the material showed signs of separating, and carbon tetrachloride then added drop by drop with stirring. Repetition of this process, followed by trituration with successive portions of carbon tetrachloride, gave minute, light yellow prisms of *tetranitrotetraphenylene* which contained carbon tetrachloride of crystallisation. On heating, evolution of carbon tetrachloride was complete at 185°, and a thick viscous liquid formed at 195—197°. This set, on cooling, to a glassy mass which did not contain halogen and was reconverted into the above crystalline condition on treatment with carbon tetrachloride (Found: C, 47·2; H, 2·1; loss at 180—190°, 24·7. $C_{24}H_{12}O_8N_4.CCl_4$ requires C, 47·0; H, 1·9; loss, 24·1%).

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Magnetochemistry.

THE NINTH LIVERSIDGE LECTURE DELIVERED ON APRIL 15TH, 1943.

By S. SUGDEN, D.Sc., F.R.S.

WHEN Council honoured me with an invitation to deliver the Ninth Liversidge Lecture I thought it would be wise to make myself acquainted with the terms of the bequest. I found it laid down that " the lectures shall not be such as are termed popular lectures dealing with generalities and giving mere reviews of their subjects nor such as are intended for the ordinary class or lecture room instruction of undergraduates." Instead they are to be " such as will primarily encourage research and stimulate the lecturer and the public to think and acquire new knowledge by research."

The short title, Magnetochemistry, which I have chosen for this lecture therefore requires a little amplification. I shall not attempt to review the whole field of chemical applications of magnetic measurements; instead I shall endeavour to outline the present position of knowledge on a few problems in which interesting advances have been made. In particular I shall try to stress some parts of the subject in which modern theory has reached a limit which it is difficult to pass but in which further experimental study of the magnetic properties of chemical substances promises to add considerably to our knowledge of the behaviour of electrons in atoms and molecules.

Nearly a century has elasped since Faraday demonstrated that most substances are affected to some extent by a magnetic field and created the nomenclature which is used to-day. The great majority of elements and compounds are feebly repelled from the poles of a magnet and are termed diamagnetic. Others are feebly attracted and are termed paramagnetic. The constant which is most conveniently used in chemical discussions is the mass susceptibility and is usually indicated by the symbol χ. For a typical diamagnetic, water, χ has the value $-0{\cdot}72 \times 10^{-6}$ c.g.s. units. Copper sulphate, a typical paramagnetic, has a susceptibility of $+6 \times 10^{-6}$ at room temperature. These figures are very much smaller than those found for the strongly magnetic metals; for instance, Swedish wrought iron in weak fields shows a mass susceptibility of more than 40 c.g.s. units. The force exerted by a magnetic field on diamagnetics and paramagnetics is consequently more than a million-fold weaker than that experienced by iron. It is therefore necessary to use specially delicate methods of measurement if the susceptibility is to be measured with any degree of precision. Care has also to be taken to protect the specimens from dust, which may cause serious errors by introducing minute particles of ferromagnetic bodies.

The methods of measurement commonly used fall into two classes. The first measures the force exerted by the field on a cylindrical specimen which is long enough to ensure that the field strength differs considerably at the two ends. Usually one end is placed in the homogeneous field between the poles of an electromagnet whilst the other end is in a field of negligible strength. This method is termed, not quite correctly, the homogeneous field method and is associated with the name of Gouy. The force can be measured by a sensitive balance or, if the specimen is liquid, by hydrostatic methods.

The Gouy method is limited in sensitivity unless a powerful electromagnet is used. For this reason Faraday and many later workers have measured the force on a small specimen in an inhomogeneous field. By inclining the pole pieces of a magnet and by other devices a field can be produced which has axial symmetry. The force on the specimen is then proportional to the product of the field strength and its gradient $H \cdot \delta H/\delta x$; since $\delta H/\delta x$ can be made large, this method gives a high sensitivity. The force can be measured by the displacement of the specimen if this is kept small by suitable control devices. An elegant modification of this method has been used by Bhatnagar and Mathur in which a light aluminium beam carries the specimen at one end and a plate of glass at the other. This plate forms part of a Rayleigh interferometer; small displacements can then be measured accurately by the movement of interference fringes.

The value of $H \cdot \delta H/\delta x$ varies rapidly with the position of the specimen with respect to the magnet and passes through a sharp maximum at the point selected for measurements. Great care has to be taken to ensure that the specimen is always placed in the correct position and that its displacement when the field is applied is very small. These difficulties can be overcome by an ingenious arrangement due to Fereday. By shaping the poles of the magnet properly and by adjusting their distance apart a region can be found in which the product of the field and its gradient is constant over a considerable volume, so that accurate positioning of the specimen is not essential. An instrument based on this principle was built by Dr. W. P. Grove at University College, London, in 1939 and gave very promising results over a wide range of temperatures.

The interpretation of magnetic data has been greatly clarified and extended by the development of modern quantum theory. The magnetic properties of atoms and molecules can now be described in terms of the number and distribution of the extra-nuclear electrons. (The spin of the nucleus makes a very small contribution which is negligible except at very low temperatures.) Diamagnetism is associated with the precession of the orbital motion of the electrons, as was shown as early as 1905 by Langevin on classical theory. Paramagnetism is due to the magnetic moment which each electron possesses in virtue of its orbital motion and its spin. These moments have to be compounded in accordance with the rules of quantum theory and cancel out for all completed sub-groups of the atom, so that it is only necessary to consider the contribution of a small number of electrons in an incomplete sub-group. It is for this reason that notable paramagnetism is found

mainly in " odd " molecules which contain an odd number of electrons and in compounds of elements of the transition series in which the atoms contain incomplete sub-groups. It has been shown by van Vleck, who has contributed largely to the fundamental theory, that in molecules another small paramagnetic contribution due to resonance between the ground state and higher states has also to be considered. The susceptibility per gram-molecule, χ_M, can thus be represented as the sum of three terms

$$\chi_M = \chi_d + \chi_\mu + \chi_R \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

The first is the diamagnetic contribution, the second the paramagnetic contribution due to the magnetic moment of the atom or molecule, and the third is the small resonance term. For most molecules which contain an even number of electrons the second term vanishes and the third term is smaller than the first so that the substance is found to be diamagnetic. Occasionally, as in potassium permanganate, the third term is greater than the first and a small paramagnetism is found which does not vary with temperature.

This qualitative agreement is satisfactory, but difficulties begin to arise when quantitative comparisons are made between theory and experiment. For atoms with a centro-symmetric field, *e.g.*, the inert gases and ions with an inert-gas structure, the second and third terms vanish so that only the first term remains and this should be given by

$$\chi_d = -\frac{Ne^2}{6mc^2} \cdot \sum_n \bar{r}^2 = -2{\cdot}84 \times 10^{-10} \sum_n \bar{r}^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where e and m are the charge and mass of the electron, N is Avogadro's number and r the distance from the nucleus. Pauling and van Vleck have deduced an expression for $\bar{r}^2$ in terms of the principal and serial quantum numbers, n and l. This is

$$\bar{r}^2 = \frac{a_0^2 n^2}{(Z-S)^2}\left\{\frac{5}{2}n^2 - \frac{3l(l+1)-1}{2}\right\} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

where $a_0 = 0{\cdot}528 \times 10^{-8}$ cm. and is the radius of the electron orbit in the ground state of the hydrogen atom; Z is the charge on the nucleus and S is a screening constant. It is this last quantity which is most difficult to estimate and which may be responsible for the poor agreement between observed and calculated values for krypton and xenon shown below.

Diamagnetism of the Inert Gases.

	He.	Ne.	A.	Kr.	Xe.
$-10^6\chi_M$, obs.	1·88—1·95	6·65—6·75	18·1—19·7	28·0—29·2	42·4—44·1
Calc. : Pauling	1·54	5·7	21·5	42	66
,, : Slater	1·68	5·7	18·9	31·7	48·0

Similar methods of calculation can be applied to ions with an inert-gas structure and again the agreement between observed and calculated figures cannot be considered good. This subject was reviewed in the *Annual Reports* for 1941 so that it need not be discussed in detail here.

For molecules the resonance term cannot be neglected. The only molecule for which even approximate numerical values can be calculated from the basic theory is that of hydrogen. Here $10^6\chi_d$ is $-4{\cdot}7$ and $10^6\chi_R$ is $+0{\cdot}5$, giving a predicted molar susceptibility of $-4{\cdot}2$ in fair agreement with the observed value of $-4{\cdot}0$. The notable feature of this calculation is the relative magnitude of the resonance term which may be associated with bond formation.

For more complex molecules it is not possible to make calculations based on fundamental theory and one has to fall back on semi-empirical methods. In a series of investigations between 1908 and 1913 Pascal measured the susceptibilities of a large number of organic compounds and showed that they could be expressed as an additive function of contributions from each atom in the molecule together with some correction terms for special structures. There have been a number of later attempts to improve and extend Pascal's method of analysis. One interesting line of attack has been opened by Gray and Cruikshank which involves an interpretation of bonding contributions in terms of screening constants, bond moments, and internuclear distances. Unfortunately, these quantities are themselves not known with any degree of precision.

The principal difficulty in the way of a more detailed analysis of the relation between diamagnetism and chemical constitution is the lack of agreement between the values found experimentally by different workers for the susceptibility of a given compound. Data for the two well-known substances, benzene and acetone, are collected in the following table.

Much greater discrepancies are found for substances which have not been studied so extensively; *e.g.*, for chlorobenzene Kido finds 68·9 against Bhatnagar's value of 72·45; for *o*- and *p*-nitrotoluene Kido's results are also some 3% lower than those obtained in Bhatnagar's laboratory.

The most self-consistent data at present available are those of Bhatnagar and his collaborators. It is clearly desirable to obtain values for a number of types of organic compound accurate to, say, one part in 500 and to have some of them checked by using different methods of measurement.

Another field in which more experimental work is needed is the variation of diamagnetism with temperature. Theoretically neither χ_d nor χ_R should be temperature-dependent in the neighbourhood of 300° K., since molecular motions should not appreciably affect the movements of the electrons. Water undoubtedly shows a temperature coefficient but this may be ascribed to changes in its degree of association. Some of the Indian workers

Susceptibility of Benzene and Acetone.

Date.	Observer.	$-10^6\chi_M$.	Date.	Observer.	$-10^6\chi_M$.
		Benzene.			
1908—1913	Pascal (corrected)	55·1	1933	Boecker	54·49
1914	Ishiwara	55·58	1934	Bhatnagar, Neogi, & Khanna	55·58
1931	Rao	54·79	1934	Bhatnagar, Mitru, & Tuli	55·64
1932	Kido	54·71	1934	Cabrera & Fahlenbrach	53·13
1933	Azim, Bhatnagar, & Mathur	55·58	1936	Trew & Spencer	55·43
1933	Rao	55·03	1936	Seely	55·45
		Acetone.			
1908—1913	Pascal (corrected)	33·7	1932	Cabrera & Madinaveitia	34·23
1931	Rao	34·53	1933	Trew & Watkins	34·52
1931	,,	34·66	1933	Rao	34·37
1931	Buchner	34·83	1935	Salceanu & Gheorgi	34·65
1932	Kido	34·43	1936	Bhatnagar, Neogi, & Mathur	33·85
1932	Fahlenbrach	33·96			

have also reported measurable changes in the diamagnetism of alkyl iodides with temperature; if this is confirmed, it will have interesting theoretical consequences.

For paramagnetic substances the predominant term in equation (1) is the second term, which may be written

$$\chi_\mu = N\mu^2\beta^2/3kT \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Here β is a convenient unit, the Bohr magneton, in which to measure the magnetic moment, μ, and has the value $0{\cdot}9174 \times 10^{-20}$ erg, gauss^{-1}. For a free atom or ion the third term in equation (1) vanishes, so that the effective moment per gram-molecule can be calculated from the susceptibility by the relation

$$\mu_{eff.} = N\sqrt{\frac{3k}{N\beta^2}} \cdot \sqrt{(\chi_M - \chi_d)T} = 2{\cdot}84\sqrt{(\chi_M - \chi_d)T} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

If equation (5) holds, the susceptibility varies inversely as the absolute temperature (Curie's law) and $\mu_{eff.}$ is independent of temperature. Equation (5) can also be applied to molecules, since in many cases the resonance term is small : in special cases a more detailed analysis is necessary. Thus, van Vleck has shown the variation of $\mu_{eff.}$ for the two paramagnetic diatomic gases, oxygen and nitric oxide, can be predicted in accurate agreement with the observed values over a wide range of temperatures.

The value of $\mu_{eff.}$ will not of course be a whole number of Bohr magnetons because of the restrictions which the quantum theory sets on the coupling of orbital and spin moments. For an isolated atom, theory shows that

$$\mu_{eff.} = g\sqrt{J(J+1)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

where g is Lande's splitting factor and J is the quantum number defining the total angular momentum of the atom. This formula gives good agreement with the data for most of the rare earths. Samarium and europium show some disagreement but a more detailed analysis by van Vleck has accounted fully on theoretical grounds for this divergence. The ions of the elements of the first transition series do not have moments predicted by formula (6); instead, they show approximate agreement with the simpler formula

$$\mu_{eff.} = \sqrt{4S(S+1)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

in which only the spin of the electrons in the incomplete 3*d* group contributes to the magnetic moment.

Van Vleck has shown that this is due to the perturbing effect of adjacent atoms on the electrons of the 3*d* group. Measurements on these ions are always made in solution or in the solid state, so these perturbations must be considered. In effect these interatomic interactions so perturb the orbital motions of the 3*d* electrons that their resultant contribution to the magnetic moment becomes zero, or, as van Vleck describes it, the orbital moment is quenched, leaving the spin moment only to contribute to the magnetism. With the ions of the rare earths the incomplete 4*f* level is screened from disturbance by a complete shell of eight electrons in the 5*sp* sub-groups and hence quenching of the orbital components does not take place.

A closer scrutiny of the data for the first transition series shows that the " spin only " formula (7) is not too closely obeyed and that the cobaltous ion and to a smaller extent the ferrous and nickelous ions give higher values of $\mu_{eff.}$ than can be accounted for on this basis. The following table gives some typical results.

Deviations from " spin only " formula.

Ion.	Substance measured.	$\mu_{eff.}$, obs.	$\mu_{eff.}$, calc.	Ion.	Substance measured.	$\mu_{eff.}$, obs.	$\mu_{eff.}$, calc.
$Mn^{..}$	$MnSO_4,4H_2O$	5·9	5·9	$Co^{..}$	$CoSO_4,7H_2O$	5·0	3·9
$Fe^{..}$	$FeSO_4,7H_2O$	5·2	4·9	$Ni^{..}$	$NiSO_4,7H_2O$	3·3	2·8

The large deviation shown by cobaltous sulphate is very much greater than can be ascribed to experimental errors. The susceptibility is proportional to the square of the magnetic moment, so the susceptibility found experimentally is 60% greater than it should be if the spin of the 3*d* electrons were alone responsible.

A preliminary survey of simple and complex cobaltous compounds was made by Dr. E. P. D. Barkworth a

few years ago (Ph.D. Thesis, London, 1938), and very considerable variations in $\mu_{eff.}$ were found which depended on the chemical character of the groups attached to the cobalt atom. Perhaps the most interesting case which was encountered was a pair of isomerides which differed considerably in their magnetic moment. When tetrapyridinocobaltous chloride ($Co\ py_4Cl_2$) is heated to 120° it loses two molecules of pyridine to leave a blue powder of the composition $Co\ py_2Cl_2$ which changes in a few hours into a violet substance of the same composition. The blue form can be regenerated by heating to 110° or by dissolving the violet form in chloroform and adding ligroin, whereupon the blue form is precipitated. The interesting fact which emerged from Barkworth's investigation is that these compounds of identical composition have notably different magnetic moments. The stable violet form gives $\mu_{eff.}$ 5·33, and the blue form $\mu_{eff.}$ 4·62. The corresponding bromide had so far only been obtained in the blue form. The diammino-complexes, $Co,2NH_3,Cl_2$ and $Co,2NH_3,Br_2$, are both known to exist in red and blue forms, but all have about the same effective moment of about 5·5. The corresponding di-iodides give a lower moment of 4·6 in both red and blue forms.

Barkworth suggests that the pyridine complexes are not true isomerides but that the colour change is associated with the change in structure

$$\underbrace{[\underset{\text{red}}{Co\ py_4}]^{++}[\underset{\text{blue}}{CoCl_4}]^{--}}_{\text{violet}} \rightleftarrows 2[\underset{\text{blue}}{Co\ py_2Cl_2}]$$

This conclusion is by no means certain but it receives some support from other lines of evidence. What does seem to be established, however, is that the coupling of the spin and orbital momenta of the 3*d* electrons in the cobaltous ion is very sensitive to perturbation by interatomic forces. A more detailed study of these cobaltous compounds is clearly needed; in particular, measurements of the effective moment over a large temperature range would give results of the greatest interest.

Most cobaltous compounds have moments which are larger than those predicted by the "spin only" formula; many other co-ordination compounds have long been known to have moments which are much lower than the predicted values. For instance, potassium ferrocyanide should have an effective moment of 5·2 and potassium nickelocyanide a moment of 2·8 if the 3*d* electrons are exerting their maximum spin; both substances are diamagnetic and have zero moment.

This behaviour has given rise to many theoretical interpretations of which the most interesting is that of Pauling (1931). This attempts to correlate the stereochemistry of the complex with the type of electronic bond and hence with the magnetism. Pauling's theory as applied to complexes derived from elements of the first transition series may be summarised as follows. (*a*) Four bonds with tetrahedral symmetry are produced by combining *s* and *p* eigenfunctions only or by combining *s*, *p*, and three *d* eigenfunctions. (*b*) Four bonds at 90° in a plane are produced by the combination of *s*, *p*, and one *d* eigenfunctions. (*c*) Six bonds directed towards the corners of an octahedron are formed by the combination of *s*, *p*, and two *d* eigenfunctions. (*d*) *d* eigenfunctions which are used in bond formation are not available for occupation by the incomplete 3*d* group of electrons.

It is this last condition which relates the magnetism of the complexes to their stereochemistry. For example, if the nickelous ion forms a planar complex by using one *d* eigenfunction the eight 3*d* electrons must occupy the remaining four *d* eigenfunctions. By Pauli's rule they must in consequence pair their spins and have zero moment. If the number of 3*d* electrons is small, then the use of *d* eigenfunctions may not cause a fall in magnetic moment. Thus in potassium chromicyanide, $K_3Cr(CN)_6$, the use of two *d* eigenfunctions to form the octahedral complex still leaves three 3*d* eigenfunctions to accommodate the three 3*d* electrons of the chromic ion. They can therefore exert their maximum spin and the chromicyanide has the same moment as the simple chromic salts.

Pauling's theory has had a large measure of success. It predicts the planar character of diamagnetic nickel complexes which has been verified by *X*-ray analysis and by the isolation of *cis-trans*-isomerides. It also predicts correctly the magnetic moment of a large number of complex compounds of the transition elements. A closer scrutiny, however, reveals a number of difficulties.

For instance a number of four-covalent complexes are known which have been shown to have a planar configuration by *X*-ray analysis but are difficult to reconcile with Pauling's theory. The cupric compounds of β-diketones fall into this group; the bivalent copper ion has 9 electrons in the 3*d* level and hence there is no vacant *d* level; yet these compounds undoubtedly have a planar configuration and also have a magnetic moment which indicates one unpaired spin. A still more difficult case is that of the beryllium compound of phthalocyanine; the beryllium atom presumably forms its bonds with 2*s* and 2*p* electrons and there is no *d* level available to take part in Pauling's planar arrangement of bonds.

Other difficulties are encountered with octahedral complexes. Many such compounds are known in which the central atom is not one of the transition elements, so the 3*d* group has not begun to be built up in the parent ion. It is possible that 3*d* levels might still be used in forming the complex but this seems doubtful from considerations of energy. Furthermore, within the transition elements the predicted fall in magnetic moment does not always take place. These points may be illustrated by the trisacetylacetonates of aluminium, iron, and cobalt.

$Al(acac)_3$.	$Fe(acac)_3$.	$Co(acac)_3$.
$\mu_{eff.} = 0$	$\mu_{eff.} = 5{\cdot}9$	$\mu_{eff.} = 0$

The aluminium compound is derived from a non-transition element and must therefore have zero moment. The ferric compound should, on Pauling's theory, have a moment of 1·83 due to one unbalanced spin; instead it has a moment which indicates five unbalanced spins, so none of the 3*d* levels is used in forming the octahedral complex. The cobaltic compound has zero spin, in agreement with the theory.

It has been suggested that compounds which do not show the expected fall in magnetism contain ionic and not covalent linkages. It is difficult to accept this view, since it implies that the ferric compound contains a type of bond which differs from that found in the aluminium and cobalt derivatives. The ferric compound can be volatilised, is soluble in non-polar solvents, and has all the properties usually associated with covalent binding.

Another example may be cited for which the attribution of ionic linkages leads to great difficulties. Bivalent iron and nickel form trisdipyridyl derivatives of the formula $[M(dipy)_3]Br_2,6H_2O$, both of which have been resolved. The iron compound has zero moment, in agreement with the Pauling theory, whereas the nickel compound has a moment of 3·0, indicating two unpaired spins. None of its 3*d* levels can therefore be used to form the octahedral arrangement of links. If the ionic explanation is to be used here, it involves the assumption that the trisdipyridyl nickel *ion* is bound together with ionic links which do not ionise in solution, whilst the closely related trisdipyridyl ferrous ion is bound by covalent linkages.

A more general theory of the magnetic behaviour of complex compounds has been developed by van Vleck (1935). He comes to the conclusion that the fall in magnetic moment can be associated with strong perturbations of the incomplete group of electrons by the close approach of other atoms. If these interatomic reactions are sufficiently powerful, they can not only quench the orbital contribution to the magnetic moment but can also bring about pairing of spins. His general argument is along the following lines. The most stable state of a free atom, *i.e.*, the one with lowest energy, is that of maximum spin. When computations are made of the energy associated with the interatomic reactions either by the Heitler–London theory or by other methods it is found that the energy associated with the atomic interactions is least when the spin is least. There are thus two opposing tendencies in a complex molecule or ion. If the interactions between the central atom and its neighbours are not too great, the tendency towards maximum spin predominates; if the interactions with the neighbouring atoms are greater, the tendency to pair spins predominates. Thus a fall in magnetic moment when a complex is formed indicates a deeper interaction or, in chemical terms, a more intimate bonding.

From this standpoint the study of the magnetism of co-ordination compounds has a renewed interest for chemistry. Already a number of observations have been made which show that even small changes in the chemical character of the outer groups can alter the coupling of electron spins in the central atom. For example, Cambi (1935) found that the nickel compound of succinimide, $Ni(su)_2,2H_2O$, has an effective moment of about 3, indicating two unpaired spins. This moment is retained if two molecules of pyridine are added to the complex, but if methylamine, ethylamine, or piperazine replaces the pyridine the moment falls to zero. Here co-ordination with primary and secondary bases appears to be more effective than co-ordination with a tertiary base. The double salt $K_2[Ni(su)_4],4H_2O$ is also found to have zero moment.

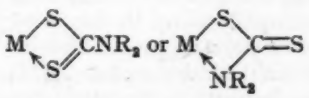
Cambi and Cagnassi have also studied a number of chelate compounds of ferrous iron. When these groups are attached at both ends by nitrogen atoms in the compounds with dipyridyl, *o*-phenanthroline, and dimethylglyoxime the magnetic moment is reduced practically to zero. If the chelate group is attached at one end through nitrogen and at the other end through oxygen, as in the compounds derived from *iso*nitroso-ketones, then a partial reduction in magnetic moment to between 1·8 and 3·6 is found.

The effect of a fixed grouping on a related series of ions is also interesting: the compounds obtained by coupling *o*-phenanthroline with bivalent manganese, iron, cobalt, and nickel have the following compositions and moments:

$Mn(phen)_3Cl_2$.	$Fe(phen)_3Br_2$.		
5·7	0		
	$Fe(phen)_3Br_2$.	$Co(phen)_3Br_2$.	$Ni(phen)_3Br_2$.
	1·1	5·1	3·0
(5·9)	(5·2)	(5·0)	(3·3)

The figures in parentheses in the bottom row are the moments of the simple hydrated metal ions. It will be seen that it is only the ferrous ion which shows any fall in magnetic moment.

Another series of interest is furnished by the substituted dithiocarbamates, which are co-ordinated either by two sulphur atoms or by sulphur and nitrogen:



A number of these have been examined by Cambi and his school, with the results shown below:

Parent ion.	Compound.	μ, obs.	μ, calc. (spin only).	Compound.	μ, obs.	μ, calc. (spin only).
Mn^{III}	$Mn(S_2CNR_2)_3$	4·8—5·0	4·9			
Fe^{III}	$Fe(S_2CNHR)_3$	5·3—6·0	5·9	$Fe(S_2CNR_2)_3$	2·5—5·4	5·9
Co^{III}	$Co(S_2CNHR)_3$	0·3	4·9	$Co(S_2CNR_2)_3$	0·4	4·9
Ni^{II}	$Ni(S_2CNHR)_2$	0·3	2·8	$Ni(S_2CNR_2)_2$	0·2—0·7	2·8

Here the tervalent manganese complex shows no reduction in magnetic moment. The iron complexes are of special interest, since they show little reduction with monosubstituted but a considerable reduction with some disubstituted dithiocarbamates. The tervalent cobalt compounds and the bivalent nickel compounds give the full reduction predicted by the Pauling theory. The ferric compounds seem to be a borderline case and would repay detailed examination over a range of temperatures and substituents.

These examples will suffice to show that the nature of the central atom and the nature of the atoms and groups attached to it both play a part in determining the magnetic moment of the complex. Further systematic study of the magnetism of co-ordination compounds is certain to lead to interesting results which will deepen and extend our knowledge of valency forces. In particular, it may be noted that most of the observations so far made have been at room temperature. A re-examination of a number of compounds of special interest over a wide range of temperature will not only confirm the values found for the magnetic moments but may lead to the discovery of systems which are so delicately balanced that change of temperature may bring about coupling or uncoupling of electronic spins.

The few topics which I have discussed form only a small part of the subject; there are many other magnetic investigations which have interesting applications to chemical problems but which lack of time forbids me even to mention. I have deliberately restricted myself to a discussion of some parts of the subject in which gaps in knowledge suggest immediate lines of attack both experimental and theoretical. This has been done in the hope that some of our younger colleagues may be tempted to undertake work in this new branch of physical chemistry. If this hope should be realised then this Ninth Liversidge Lecture will in some measure have fulfilled the wishes expressed by its founder.

OBITUARY NOTICES.

JOHN WILLIAM BLAGDEN.

1876—1942.

Dr. J. W. Blagden died on November 28th at his home in Loughton, Essex, in his 67th year. He came from a scientific family, and was educated at Dulwich and Emmanuel College, and took an Honours degree in Natural Science in 1895. Proceeding to Würzberg, he worked under Hantzsch, obtained his Ph.D., and after a period as Senior Demonstrator in Electro-Chemistry at Darmstadt, entered industry with C. F. Bohringer und Söhne as Research Chemist and Departmental Manager. He worked on electrolytic oxidations and reductions and later became interested in the then new field of catalytic hydrogenations. In this, particularly in its applications to industry, he was one of the pioneer workers. The outbreak of war in 1914 put an end to his work in Germany. During the period of hostilities he was an internee at Ruhleben, where as the leader of a group he endeavoured to continue scientific work, an effort typical of a man who refused to be dismayed by difficulties. At the end of the war he returned to England and sought an opportunity to continue his work on catalysis. He joined Messrs. Howards and Sons, Ltd., in 1919 and undertook the organisation and direction of a newly formed Research Department. The range of new products manufactured by the firm during the last twenty years is a tribute to the energy and skill with which he pursued the task. In 1929 he was elected a Director of the Company.

He was a Fellow of the Chemical Society and of the Institute of Chemistry, and a Member of the Society of Chemical Industry. He served on a number of Committees, among which may be mentioned the Pharmacopœia Commission, the Essential Oils Committee of the Imperial Institute, Chemical Committees of the British Standards Institution, and the Patents Committee of the Association of British Chemical Manufacturers.

He had little desire or time for publication, and apart from a few relatively minor papers the record of his work exists in patents and in the technical achievements of the laboratory under his direction.

Blagden was always accessible to members of his staff, and devoted much of his time to their encouragement and welfare.

He felt that Industry owed a debt to the Universities, and was pleased to acknowledge this by both material and technical assistance whenever possible.

G. C. Clark.

ARTHUR HARDEN*

1865—1940.

Arthur Harden died on 17 June 1940 at his home at Bourne End.

Harden was born in Manchester on 12 October 1865. His father was Albert Tyas Harden, a Manchester business man, who had married Miss Eliza MacAlister, of Paisley. He was the only son, but there were several sisters, and the family was brought up in an austere nonconformist atmosphere, abjuring the theatre and regarding Christmas as a pagan festival. At the age of seven years he was sent to a private school kept by Dr. Ernest Adam in Victoria Park, Manchester, and four years later he went on to the Tettenhall College in Staffordshire, where he stayed until he was sixteen. In January 1882 he entered the Owen's College, Manchester, and studied chemistry under Professor Roscoe, then at the height of his fame as a teacher. In 1885 he graduated in the Victoria University with first-class honours in chemistry. A year later he was awarded the Dalton scholarship.

It was J. B. Cohen, to whose stimulating teaching Harden doubtless owed much, who suggested the subject of his first research, " The action of silicon tetrachloride on aromatic amide-compounds," and in the following year the results of this investigation were published in the *Transactions of the Chemical Society* (1886). From Manchester he proceeded to Erlangen, and under the direction of Otto Fischer prepared β-nitroso-naphthylamine and investigated its properties. Having been awarded the degree of Ph.D., he returned to Manchester and there became first junior and then senior lecturer and demonstrator under Professor H. B. Dixon, who had meantime succeeded Sir Henry Roscoe as professor of Chemistry. During part of this time Harden shared the laboratory teaching with Hartog (later Sir Philip), and for some years he lectured to the honours students on the history of chemistry, a subject in which he was greatly interested. This interest was shared by Roscoe: together they studied the note-books of Dalton and arrived at the conclusion that it was Dalton's investigation of the relations governing the diffusion of gases that led him to formulate his atomic principles. They published their views in 1897 in the *Philosophical Magazine* and in the *Zeitschrift für physikalische Chemie*, and afterwards in a book entitled *A new view of the genesis of Dalton's atomic theory*. Harden's interest in Dalton endured, and in 1915, with H. F. Coward, he communicated to the Manchester Literary and Philosophical Society a description with comments of some of the lecture sheets with which Dalton used to illustrate his lectures and which had recently been discovered in the rooms of the Society.

In 1895 Roscoe and Harden collaborated in writing a textbook to fulfil the needs of students for the higher

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South Kensington examination. In collaboration with F. C. Garrett, Harden also wrote a book on *Practical organic chemistry*, designed to supplement Perkin's *Organic chemistry*, and also undertook with Colman the work of revising and editing a new edition of Roscoe and Schorlemmer's *Treatise on inorganic chemistry*. All this literary activity served as valuable training for the editorial work which was to occupy so much of the time of his later life.

During these nine years in Manchester, Harden seems to have been chiefly interested in his teaching and literary work. He published only two papers based on experimental work; both appeared in the *Transactions of the Manchester Literary and Philosophical Society*, one with W. Haldane Gee on a new form of stereometer (in 1891) and the other on the composition of some iron and bronze implements which had been found by Professor Flinders Petrie in 1897. He began experiments on the combination of carbon monoxide and chlorine, with a view to studying the phenomenon of photo-chemical induction, a subject in which Dixon was especially interested: this work was carried out in collaboration with Dyson, but the results were not published until 1903, six years after Harden had left Manchester. It seems, therefore, that up to the time he left Manchester, at the age of thirty-two, Harden had not manifested an overpowering desire for scientific research. He had applied for the posts of Principal at the Wandsworth Technical Institute and of Inspector under the Science and Arts Department at South Kensington: fortunately both these applications were unsuccessful.

In 1897 Harden was appointed to succeed Joseph Lunt as chemist to the Jenner Institute of Preventive Medicine in London. The choice was made largely on the grounds that he had a wide knowledge of chemistry and had proved himself to be a successful teacher. At that time the institute provided instruction in bacteriology and chemistry for medical practitioners desiring to equip themselves to become medical Officers of Health. Harden was responsible for the chemical course which was mostly concerned with the analysis of water and foods.

After a few years, however, the London medical schools introduced special teaching for those wishing to qualify for a diploma in public health, so the classes at the Jenner Institute were discontinued and Harden was henceforth free to devote his whole time to research.

There were then two chemical departments at the institute (which had meantime changed its name to Lister Institute), one called Chemical was under Harden, the other Biochemical was presided over by Hedin, with Leathes second in command. In 1905 Hedin left and Leathes was shortly afterwards invited to occupy a chair of pathological chemistry at Toronto, so the two departments were fused and Harden was placed in charge. In 1912, in recognition of his outstanding work on bacterial chemistry and alcoholic fermentation, he was made Professor of Biochemistry in the University of London.

At the Jenner Institute Harden made his first contacts with biology. His senior colleague was Alan Macfadyen, who was in charge of the bacteriological department, and it was at the latter's suggestion that in 1898 he embarked upon an investigation of the fermentation of sugars by bacteria. The original object of this was to find diagnostic means for the differentiation of varieties of *Bacillus coli*, but Harden gave a wider scope to the research. He kept a carbon balance sheet by determining the amounts of the various products. Incidentally, he discovered several substances not known at that time to be formed by the bacterial decomposition of sugars. Several products of the fermentation of *B. coli* had been identified earlier, but Harden was the first to construct a picture of the breakdown process as a whole. His first paper on the action of *B. coli communis* and allied organisms on sugars appeared in 1899 and a more extensive account in 1901. In this he compared the results of the fermentation of glucose with that of other hexoses and pentoses and on mannitol and glycerol. He showed that acetic acid and ethyl alcohol were formed in equimolecular proportions and that the two terminal alcohol groups were the source of them. In 1905 he published similar observations upon the action of *B. lactis aerogenes*. With this organism the amount of acetic acid and alcohol formed was not equal. One-third of the glucose fermented could not at first be accounted for, and the deficit was found partly in the form of acetyl methylcarbinol and partly as the reduced form as butylene glycol. These two substances had not hitherto been known to be products of bacterial action on sugars. The former, he found, was the substance responsible for the colour reaction of Voges and Proskauer used as a diagnostic criterion by bacteriologists. Further papers on bacterial chemistry with Norris and with Penfold appeared in 1912.

Harden's work on bacterial chemistry did not attract as much attention at the time as its merit justified. The bacteriologists did not appreciate what it was about, and it was too far removed from the interests of all but a few chemists. It was, indeed, pioneer work and formed the basis on which many interesting researches into bacterial metabolism have subsequently been built.

Harden commenced to work on bacterial chemistry in 1897, and in 1900 he started his researches on alcoholic fermentation, in the course of which he made some notable discoveries which will remain associated with his name. These two lines of research proceeded simultaneously for some ten years, but for simplicity of description they are dealt with separately.

Alcoholic Fermentation

Harden derived his interest in alcoholic fermentation also from his bacteriological colleague, Alan Macfadyen, who, like most microbiologists, was impressed with Buchner's discovery that the cell-free juice expressed from yeast would ferment sugar.

The belief that alcoholic fermentation required the operation of the intact living cell having been superseded, a new experimental method for its study had been found. The first crude preparations of zymase had, however,

two drawbacks: (1) yeast juice produced a considerable amount of alcohol and CO_2 without the addition of sugar; (2) it rapidly lost its power to ferment. Harden's first researches were concerned with discovering the cause of these disturbing phenomena with a view to overcoming their hindrance to quantitative experimentation. Autofermentation he found to be due to the action of the enzyme upon the glycogen expressed from the yeast cells and that it ceased when this supply was exhausted. Deterioration in fermentative activity, he concluded, was brought about by destruction of the zymase by the proteolytic enzyme also present in the expressed juice, for by the time autolysis was advanced its power to ferment had disappeared.

It was whilst engaged on these investigations, neither of which had direct significance for the understanding of the chemistry of the transformation of sugar into alcohol and CO_2, that he made two fundamental discoveries. The first was that the enzyme zymase would not convert sugar into alcohol unless another substance which functioned as a co-ferment was present. The second was that the presence of phosphate was also necessary.

It is doubtful whether Harden would have made the advances he did had he not early abandoned the gravimetric measurement of CO_2 produced, which had been used by Buchner and others, and adopted a volumetric one. The simple method he substituted enabled him to make frequent determinations of the CO_2 evolved and thereby study the progress of the reaction at ten-minute intervals, if necessary, throughout the period of the experiment.

In his studies on alcoholic fermentation Harden was powerfully supported by a number of able pupils and collaborators which he had attracted to his laboratory. Notable amongst these were W. J. Young, now Professor of Biochemistry in the University of Melbourne; R. Robison, who succeeded him; Lord Henley; and Miss Macfarlane, who still carries on the good work at the Lister Institute. These collaborations with younger folk were happy and fertile. Harden's strong points as an investigator were his wide knowledge of chemistry and his capacity to analyse the results of an experiment dispassionately and define accurately what interpretations were justifiable. He was a superb critic but unduly suspicious of adventures into the realm of imagination.

The Co-Enzyme

How Harden came to discover co-zymase and the rôle of phosphates in alcoholic fermentation makes an interesting story which indicates his measure as an investigator.

For some reason or another, which is not now material, he added boiled autolysed yeast juice to a mixture of zymase and sugar solution and found that the rate of fermentation was thereby greatly increased. At first he attributed this to some restraining influence on the proteolytic enzyme, always present with zymase, which he had previously found to be inhibited by horse serum and other proteins. This explanation had, however, to be put aside after appropriate experiments, and he was faced with the fact that yeast juice contained, in addition to zymase and a proteolytic enzyme, a substance stable to boiling which favourably influenced fermentation. Additional study of its properties showed that it was thermostable, dialysable through parchment paper, destroyed by incineration and precipitated by 75 per cent. alcohol.

Further than this he could not advance until some satisfactory method of separating the unknown substance from zymase was found. Dialysis was too slow, for meantime the zymase was being destroyed by the proteolytic enzyme.

The separation was ultimately achieved by employing a Martin gelatin filter, by means of which a separation could be effected in a few hours, the zymase remaining on the outside of the filter and the activating substance passing into the filtrate. Neither the redissolved residue nor the filtrate alone would ferment, but when the two were mixed fermentation occurred at about the original rate. For this necessary adjunct to fermentation by zymase the name co-ferment was suggested.

Harden did not discover more as to its nature beyond finding that it contained phosphoric acid in its make-up, nor as to what stage in the conversion of sugar to alcohol it was operative, but he did hazard the suggestion that as it contained phosphate it might be involved in the phosphorylation of the hexose, which he had also discovered to be an essential stage in the process and which will be dealt with immediately. The constitution of the co-ferment was ascertained by von Euler some years afterwards. He showed that it was a complex nucleotide containing adenine, nicotinic acid and rhibose, and proposed the name co-zymase for it.

The Essential Rôle of Phosphates

Experiments with boiled yeast juice also led Harden to discover the necessity for phosphates in fermentation by zymase. As just mentioned, the co-ferment was destroyed by incineration, or could be precipitated by 75 per cent. alcohol, but the ash or the filtrate after precipitation still produced a temporary increase in the production of CO_2.

This result led to the trial of the addition of potassium phosphate. The result was startling. In fifteen minutes the rate of production of CO_2 had increased sevenfold. It then fell as rapidly to the original rate. It was much as if an acid had been added to a solution of bicarbonate, for the amount of CO_2 produced was equimolecular with the HPO_4 added.

This and the fact that it could be repeated, indicated that the phosphate had somehow become fixed. This was proved by showing that the phosphate was no longer precipitable by magnesia mixture. Harden and Young subsequently found that it had combined with the sugar to form a hexosediphosphate. This ester was subsequently isolated and identified by Young.

The study of the fermentation of mannose and fructose threw further light on the constitution of the hexosephosphate. Mannose was fermented as rapidly and fructose more rapidly than glucose. The rate of fermentation of all three sugars was accelerated by phosphate, but that of fructose was double that of glucose or mannose and it was much less rapidly inhibited by the further addition of phosphate. The hexosediphosphate prepared from these three sugars was identical and the quantitative relations were expressed by the same equations. It was suggested that the hexosephosphate was probably derived from the enolic form common to the three sugars, though the possibility that the two molecules of sugar were broken down into smaller groups and hexaphosphate resynthesized from these was considered possible.

As fermentation by the intact yeast cell proceeds at a uniform and enhanced rate compared with that by zymase, it was clear that the bound phosphate must be liberated as quickly as it was formed. Harden and Young soon discovered that yeast contained a phosphatase which hydrolysed hexosediphosphate, so presumably this accounted for its non-accumulation and they thought that this mechanism must be defective in the case of yeast juice and so account for the relatively slow rate of fermentation.

Experiments were therefore made in which phosphatase prepared from yeast was added to a zymase preparation in which fermentation had nearly ceased after the spurt occasioned by addition of phosphate. The addition of phosphatase caused the fermentation rate to increase much as if a new dose of phosphate had been added, but it could not be maintained, although the increased rate endured longer than when phosphate was added. The conditions obtaining in the living cell could not be completely imitated in zymase fermentation, as for some reason the added phosphatase lost its activity, but it could be done indirectly by the addition of arsenates—a result no one could have foreseen. As Harden said in his Nobel lecture, " this observation was the undeserved reward for thinking chemically about a biochemical problem, a dangerous thing to do." As in many chemical reactions arsenates behave like phosphates, out of curiosity he tried whether they could replace them in alcoholic fermentation. This did not occur. The addition did indeed increase the rate of fermentation, but the effect was not sudden and temporary as that of phosphate, but slower and more lasting. Arsenates behaved in an entirely different way, apparently by preventing the destruction of phosphatase.

Harden and Young suggested that their observations indicated that fermentation could be illustrated by two equations :

(1) $2C_6H_{12}O_6 + 2Na_2HPO_4 = C_6H_{10}O_4(PO_4Na_2)_2 + 2H_2O + 2CO_2 + 2C_2H_6O$;
(2) $C_6H_{10}O_4(PO_4Na_2)_2 + 2H_2O = C_6H_{12}O_6 + 2Na_2HPO_4$.

the first representing the evolution of CO_2 and production of alcohol and accumulation of phosphoric ester; the second the hydrolysis of the ester with liberation of a hexose and mineral phosphate.

In the presence of arsenate, equation (2) proceeds sufficiently rapidly to supply phosphate so that equation (1) proceeds with maximal velocity.

In 1914 Harden and Robison discovered that the diphosphate was not the only phosphate ester formed during fermentation, but that hexosemonophosphate was also present. The relative amount of these two phosphates varied from 80 per cent. of one or the other according to circumstances. Nevertheless the ratio of CO_2 evolved to PO_4 esterified remained constant and approximately unity. What were the conditions for the preferential formation of the mono- or diphosphate could not be discovered. Harden was convinced that complete reaction of some sort took place as the result of which two phosphate groups were introduced into certain sugar molecules—either into the same molecule or one each into two different molecules—and this induced the decomposition of another molecule of the carbohydrate. Robison and he showed that the monophosphate reacted with a further quantity of phosphate and was accompanied by an increased production of CO_2.

Harden recognized that the only quantitative relation that his experiments had established was that during the course of fermentation by yeast juice, or by dried yeast, for every one molecule of phosphate esterified approximately one molecule of CO_2 was evolved.

This view and its expression in the form of the equations (1) and (2) above truthfully expressed the beginning and the end of the reactions and the final result. The intermediate stages, however, still remained obscure until Meyerhof and his colleagues established that the monophosphate was the first product, and that hexosediphosphate was formed by the further phosphorylation of the monophosphate, which was then converted into two molecules of triosephosphate, that the course of the breakdown began to be clearer.

Henceforth, with the exception of four years during the late war, most of Harden's research activities were devoted to attempts to elucidate the reactions involved in fermentation and the rôles played by zymase, cozymase and phosphates respectively. It was a period of controversy, with experiment and counter-experiment, and it is difficult to make a connected story of Harden's contributions, for the significance of individual experiments is not easily apparent.

Buchner's suggestion that lactic acid was an intermediate in sugar fermentation was rejected by Harden, a view in which Buchner eventually concurred, though unfortunately not before it had been widely accepted by biologists. Iwanoff claimed to have isolated a triosephosphate giving an osazone (m. p. 128°) similar to one prepared by Lebedeff from the oxidation products of glycerol. According to Iwanoff the formation of hexosephosphate preceded the alcoholic fermentation and the amount of fermentation should be found proportional to the amount of hexosephosphate decomposed. Harden did not at first accept this view, but the existence of Iwanoff's triosephosphoric acid was confirmed in 1907 by his colleague Young, who isolated it as a lead salt.

No attempt seems, however, to have been made by Harden to introduce the triosephosphate into his equations. It was shown by Macfarlane, working in Harden's laboratory, that an autolysed solution of dried yeast which was incapable of fermenting glucose even after the addition of the co-enzyme could decompose hexosediphosphate, producing inorganic phosphate and CO_2. The existence of a definite phosphatase acting on the hexosediphosphate was therefore established. When co-enzyme and arsenate were added the decomposition of the hexosediphosphate was extremely rapid, and CO_2 and inorganic phosphate appeared as final products of the decomposition. It was the breakdown of the hexosediphosphate that was stimulated by the presence of arsenate.

Eighty per cent. of the great fall in fermentative activity which occurred when fresh yeast was converted into yeast juice was found to occur during the process of grinding, and it was during the same period that the yeast acquired the power of responding to phosphates. It was therefore concluded that the phosphatase was destroyed.

The nature of the enzymes in yeast and their method of action was investigated by Harden and his colleagues in a long series of researches.

He confirmed Neuberg's discovery of the existence of the enzyme carboxylase in dried yeast, which split pyruvic acid into CO_2 and acetaldehyde and showed that as this reaction took place after the co-enzyme had been completely washed away, it was independent of the presence of the co-enzyme. The reducing power of zymin or dried yeast on the other hand was removed by washing, but could be restored by the addition of the boiled washings. Certain aldehyes also restored the reducing power and therefore had the property of acting as oxygen acceptors. Similar experiments carried out with washed rabbit's muscle showed that here the addition of either lactic acid or acetaldehyde restored the reducing power. Evidence was also obtained of the existence of a peroxidase in fresh yeast. The peroxidase activity was lost on drying for seventeen hours at 37° C. but was again detected after the dried yeast had been washed. The catalase activity was not affected by washing, whereas invertase, but not maltase, was removed by washing the dried yeast. Harden also showed that the presence of inorganic salts exercised a marked influence on the autofermentation of yeast; all dissolved substances which plasmolysed the yeast cell caused also a large increase in the rate of autofermentation. Washed zymin in the presence of a suitable concentration of sodium phosphate was activated by potassium pyruvate, but not by acetaldehyde; if, however, the sodium phosphate were replaced by the potassium or ammonium salt, acetaldehyde also acted as an activator. There was therefore a specific salt effect, the sodium ion acting differently to the potassium or ammonium ion. On the other hand, when the fermentation of fructose or glucose by dried yeast preparations was examined it was found to be equally depressed by the addition of sodium or potassium chloride and the effect of the sulphates was somewhat greater. This action was shown to affect the rate of action of the hexaphosphatase but not that of the carboxylase. Potassium phosphate was also shown to produce a depressing effect, but in this case, unlike that of the sulphate, the depressing effect could be largely counteracted by the addition of acetaldehyde to the solution.

Application of Harden's Discoveries to Bacterial Metabolism and Animal Physiology

After Harden's discovery of the co-enzyme and of the important part played by phosphates and phosphoric esters in alcoholic fermentation, it was soon discovered by various workers that phosphorylation provided the clue to many other biological phenomena.

Harden's earlier work on bacterial metabolism was done before his studies of fermentation by yeast had revealed the importance of phosphorylation as a factor in the latter. He did not himself undertake experiments to decide whether it would prove of equal importance in the former. During recent years, however, the work of many investigators has shown that this is actually the case. During glycolysis under the influence of *Bacillus coli*, for instance, the bacterium which had been Harden's chief concern, the main stages of breakdown are undergone by phosphorylated molecules. In brief, the following sequence occurs: Hexosediphosphate yields phosphoglyceraldehyde. By dismutation this yields 1-glycerolphosphate and phosphoglyceric acid. Of these two products the former breaks down into ethyl alcohol and free phosphates, while the latter yields phosphopyruvic acid. In aerobic conditions this last product breaks down into free phosphate, acetic acid and CO_2; while anaerobically formic acid takes the place of CO_2. The liberated inorganic phosphoric acid becomes available for the further formation of the original hexosephosphate.

An astonishing degree of similarity was also found to exist between almost every detail of the production of lactic acid by the muscle enyzmes and of alcohol by the yeast enzymes, which extended to the identity of the phosphoric esters concerned, the accumulation of ester under similar conditions and even to the effect of arsenates on the process.

In an aqueous extract of muscle glycolysis proceeds, but neither glycogen nor glucose molecules suffer change until they have become associated with phosphoric acid as its esters. The various simpler molecules which represent intermediate stages in the sequence of anaerobic reactions which ultimately liberate the available energy of the original carbohydrate molecules also undergo esterification or suffer substitution by the phosphoryl radicle. Of fundamental importance in maintaining the sequence of events is the circumstance that the phosphoryl radicle is readily transferable from one molecule to another, from a donator of phosphate to a receptor. Such transference leads to the coupling of reactions, and in particular cases may result in the actual transference of energy from exothermic to endothermic events. The study of these phenomena gained much in interest when it was shown (in the School of Parnas in Lwow; in Cambridge by D. M. Needham and van Heyningen, and by Meyerhof and collaborators) that the progress of the reactions depends in general on the presence of adenylic

acid in muscle. It has been shown, for instance, that the events which yield the lactic acid of anaerobic glycolysis involve an oxido-reduction reaction strongly exothermic in character. It comprises an oxidation of glyceric aldehyde and a simultaneous reduction of pyruvic acid. In the absence of either inorganic phosphate or adenylic acid, however, this reaction does not occur. In their presence it actively proceeds, yielding the energy for a strongly endothermic reaction, namely, the esterification of adenylic acid to adenose pyrophosphate. This latter is a compound with high free energy content, exerting indispensable functions both as a donator and receptor of the phosphoryl ion. On hydrolysis its energy is liberated and it is indeed legitimate to speak of the energy of phosphate bonds. It would seem that the myosin of the muscle fibres itself catalyses the dephosphorylation of this pyrophosphate. It is likely, therefore, that a local breakdown in the fibres yields, or contributes to, the energy necessary for muscular contraction. The pyrophosphate structure is then reconstituted by transference of phosphoryl from yet another muscle constituent, namely creatin phosphate, which may be looked upon as a phosphate accumulator.

It is of interest to find that another aspect of Harden's work, namely, his demonstration of the effect of arsenate, has greatly assisted towards an understanding of the mechanism of the reactions just described. At the same time, the muscle studies have more fully explained the effect of arsenic on alcoholic fermentation.

This brief account omits many details now known concerning the events in muscle, nothing, for instance, being said about the enzymes which catalyse the reactions. It should make clear, however, the remarkable lines on which the influence of phosphates is exerted. Recognition of the significance of Harden's discoveries constituted a turning point in the history of muscle chemistry.

Robison's significant observations on ossification were also a direct outcome of his association with Harden in work on fermentation. The question why calcium and phosphorus should be normally deposited in cartilage that was about to ossify and only pathologically elsewhere remained unanswered. It occurred to Robison that there must exist in ossifying cartilage some conditions producing supersaturation of calcium and phosphate ions, and that one way in which this might occur would be by the decomposition locally of phosphoric esters in the blood supplied, supposing the tissue contained phosphatase. On examination it was found that cartilage about to ossify did indeed contain a phosphatase, whereas it was absent from the cartilages of the patella and of the trachea which do not undergo ossification.

When war broke out in 1914, the Lister Institute soon became depleted of most of its male staff and research students, while those who remained concerned themselves with problems having some relationship to our state of belligerency. Harden was left in charge of the institute during the absence of the director on military service. Wanting to devote himself to some subject which would contribute to the war effort, he abandoned his researches on alcoholic fermentation for five years and investigated two of the then known accessory food factors or vitamins, lack of which there was good reason to believe was responsible for the diseases beri-beri and scurvy respectively. Both of these diseases had occurred amongst our troops at outposts in Asia and Africa, where, either from difficulty of transport or from being beleaguered, they had to subsist on a restricted diet. The study of accessory food factors had assumed military importance and the exploration of means to effectively supplement rations had become a matter of some urgency. These investigations were not confined to the application of existing knowledge, but involved work of a fundamental character as the chemical composition of the two factors was unknown and their properties but little known. In carrying them out, Harden had valuable assistance from S. S. Zilva, then a research student in his department.

They proved that neither 2-hydroxypyridine nor adenine was the active agent which cured beri-beri as had been stated. The two water-soluble factors were differentiated by the use of fuller's-earth, which absorbed the anti-neuritic but not the antiscorbutic factor. The former was shown to be essential for the growth of frogs. The fruit-fly *Drosophila* was found to need the antineuritic but not the antiscorbutic. The biological synthesis of the antineuritic by yeast was established.

Holst's experiments showing that scurvy was a dietary disease due to a deficiency of a specific substance were confirmed, and a step towards the separation of this principle was achieved by removal of the organic acids, carbohydrates and protein from lemon juice and the production of a residue of much increased potency. In collaboration with Dr. Still, the superiority of this concentrated product in the treatment of infantile scurvy was demonstrated.

The susceptibility of the antiscorbutic principle to adverse influences was studied and its particular sensitivity to alkalies discovered. The effect of heating and storage at different temperatures on the potency of fruit juices was investigated and results of immediate practical importance obtained.

Scurvy was produced in monkeys by a scorbutic diet and the relative antiscorbutic requirements of this animal ascertained. Rats, on the other hand, did not develop scurvy.

As, in older days, fresh ale had a reputation for antiscorbutic properties, and yeast being one of the richest sources of the antineuritic principle, stout, ale and fined beer were tested, but both principles were found lacking in all of them.

Although research on nutrition had not been Harden's métier, he adapted himself to work in this new field with conspicuous success, and the results achieved make a goodly contribution to the early development of our knowledge of accessory food factors. Incidentally, it led to his acquiring a wide knowledge of the subject, which was used to good effect in editing the three editions of *Present knowledge of accessory food factors*, published by the Medical Research Council in 1919, 1924 and 1932.

Recognition of the importance of Harden's researches came from many quarters. In 1909 he was elected a

Fellow of the Royal Society, and in 1935 was awarded the Davy Medal by the Society. He served on its council from 1921 to 1923. In 1929 he shared the Nobel prize for chemistry with von Euler. The Universities of Manchester, Liverpool and Athens conferred honorary degrees upon him, and the Kaiserlich Leopold Deutsche Akadamie der Naturforscher of Halle elected him to its membership. In 1936 he received the honour of knighthood.

An account of Harden's scientific career would be incomplete without some reference to his teaching ability and his literary activities. In the early days at Manchester his powers as a teacher were recognized. At the Lister Institute he had little opportunity to exercise this gift in which he found pleasure, but for many years he devoted one evening a week to a class in microbiology at the Sir John Cass Institute, and for a while gave a course of evening lectures on biochemistry at the Chelsea Polytechnic. In both he took the greatest pains and interest.

Literary work took up a good deal of his time. Beside the very onerous work of editing the *Biochemical Journal*, he wrote the article on " Fermentation " for Thorpe's *Dictionary of chemistry* and the section on yeast enzymes for Oppenheimer-Pincussen's *Die Fermente und ihre Wirkungen* and the section on alcoholic fermentation, " The early stages of fermentation in the yeast cell," for *Die Ergebnisse der Enzyme Forschung*, and he was the author of the monograph on Alcoholic Fermentation in the Longmans series of *Biochemical monographs*, of which four editions have already appeared.

Harden's outstanding qualities as an investigator were clarity of mind, precision of observation and a capacity to analyse dispassionately the results of an experiment and define their significance. He mistrusted the use of his imagination beyond a few paces in advance of the facts. Had he exercised less restraint, he might have gone further; as it was, he had little to withdraw.

His personal relations with his fellow-men were controlled by an uncommon reserve. His attitude to most was that of a benevolent neutral. Few reached intimacy with him, but those who did penetrate his protective covering found in him a lovable and staunch friend.

He was generally cheerful, but never exuberant and suspicious of exuberance in others. He had a dry humour and was capable of unexpected flashes of wit. It was not his habit to offer his opinions unasked, but his advice and help were freely given if called for.

He had a very equable temperament and said that he had never known great elation or great depression. The uncommon control he exercised over his emotions was perhaps attributable in part to the severe puritanical discipline of his early life. Whatever the cause, his judgment was little influenced by them, so that he was pre-eminently a fair man. He would have made a great judge.

In 1890 he married Georgina Bridge, of Christchurch, New Zealand. His wife died in 1928; they had no children.

In 1930 he retired from his professorship at the Lister Institute, but continued to come every day to his laboratory, carrying on experimental work and correcting proofs for the *Biochemical Journal* until a year or two before his death. Towards the end of his life he suffered from a progressive nervous disease from which release came before it had become too irksome and while he still enjoyed his garden, his books and his interest in science.

F. G. Hopkins.
C. J. Martin.

ALFRED HUTCHINSON.

1866—1938.

Alfred Hutchinson, who died on August 19th, 1938, at the age of 72, was a native of Middlesbrough, and throughout his life was associated with the iron and steel industry of that district. His father, Mr. Thomas C. Hutchinson, managing director of the Skinningrove Iron Co. Ltd., was well known as an original and enterprising industrialist, who was responsible for many innovations in iron and steel plant. His son passed through the local High School and at the age of 15 went to Leys School, Cambridge. He took a London B.Sc. degree, intending to enter the family business, but becoming interested in biology and medicine, he returned to Cambridge, and gained a Major Scholarship at Trinity College. He obtained first class honours in the Natural Science Tripos and took a post as junior science master in his old school. With the head master, Dr. C. W. Kimmins, he built up a Science VI form, and among their pupils were Sir Joseph Barcroft and Sir Henry Dale. In 1894 he left Cambridge and entered the Skinningrove works, being first secretary, then blast-furnace manager, and on his father's death managing director and later chairman. He was concerned in a number of technical improvements, and contributed papers to the Iron and Steel Institute on questions relating to metallurgical fuels. In that Institute he took an active part, and held the Presidency for the year 1937—38. He became a Fellow of the Chemical Society in 1894. He was keenly interested in all questions relating to fuel, iron and steel, and his wide scientific culture stood him in good stead. He was greatly respected in the industry, and also in the county and municipal life of the North Riding, with which he was connected for so long.

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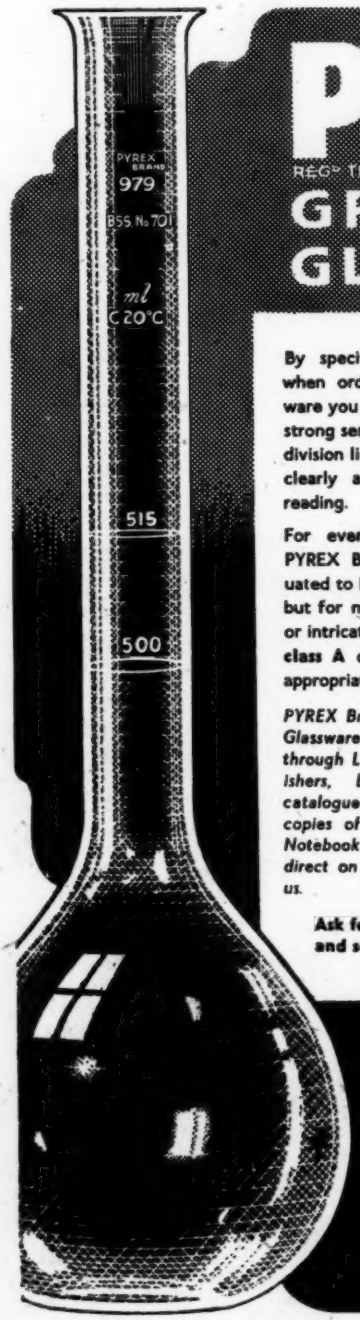

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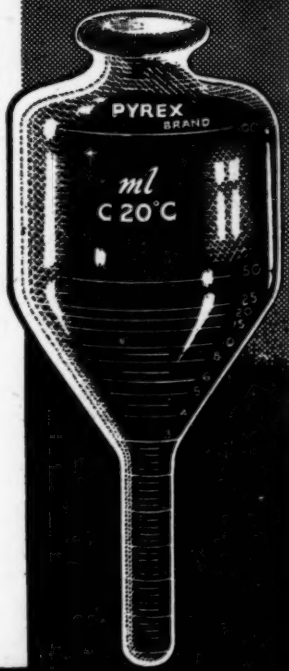

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